

BIOCHEMICAL AND PHARMACOLOGICAL STUDIES  
ON BLOOD PLATELETS AND ERYTHROCYTES  
IN AFFECTIVE ILLNESS

by

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"Though this be madness, yet there  
is method in't."

Hamlet II . ii . 211

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STATEMENT IN TERMS OF  
Ph.D. REGULATION 2.4.11 OF THE  
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Certain parts of the work described in this thesis have already been published;

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STATEMENT IN TERMS OF  
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I declare that this thesis was totally composed by myself and that all work described herein was initiated by myself, with one exception. Dr. L.J. Whalley was responsible for setting up the ECT study which is described in this manuscript.

All the experimental work was carried out by myself with the following exceptions:

- i) patient assessment and venesection was performed  
by doctors working at the Royal Edinburgh Hospital
- ii) plasma lithium levels were estimated by Mrs. H.  
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## ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine 5'-triphosphate
ATPase	adenosine triphosphatase
$^{\circ}\text{C}$	degrees centigrade
$\text{Ca}^{2+}$ - ATPase	calcium stimulated, magnesium dependant ATPase
$\text{CaCl}_2$	calcium chloride
$\text{Ca}^{2+} + \text{Mg}^{2+}$ - ATPase	total magnesium dependant and calcium stimulated, magnesium dependant ATPase
cAMP	cyclic AMP (adenosine 3',5'-monophosphate)
Ci	Curie
CNS	central nervous system
COMT	catechol-O-methyltransferase
CSF	cerebrospinal fluid
DA	dopamine (3-hydroxytyramine)
DNP	dinitrophenol
DOPAC	3,4-dihydroxyphenylacetic acid
dpm	disintegrations per minute
DTNB	dithio-bis-nitrobenzoic acid
E	activation energy
ECT	electroconvulsive therapy
EDTA	ethylenediaminetetracetic acid
fmole	femto-mole
g	gauge, gramme or gravitational force
HCl	hydrochloric acid
hr	hour
HRS	Hamilton Rating Scale
5-HT	5-hydroxytryptamine (serotonin)
HVA	homovanillic acid (3-methoxy-4-hydroxy-phenylacetic acid)
i.u.	international unit
i.v.	intravenous
Kcal	kilocalorie
KCl	potassium chloride
$K_D$	dissociation constant
$K_m$	Michaelis Menton constant

$\log_{10}$	logarithm to the base 10
M	Molar
MAO	monoamine oxidase
mCi	milliCurie
$\text{Mg}^{2+}$ - ATPase	magnesium dependant ATPase
$\text{MgCl}_2$	magnesium chloride
$\text{MgSO}_4$	magnesium sulphate
MHPG	3-methoxy-4-hydroxyphenylglycol
mins	minutes
ml	millilitre
mM	milliMolar
mmol	millimole
MRC	Medical Research Council
MSE	Measuring and Scientific Equipment Ltd
NA	noradrenaline
$\text{Na}_2\text{ATP}$	disodium adenosine 5'-triphosphate
$\text{NaCl}$	sodium chloride
$\text{NaF}$	sodium fluoride
$\text{Na}^+ + \text{K}^+$ - ATPase	sodium and potassium stimulated, magnesium dependant ATPase
$\text{NaOH}$	sodium hydroxide
nm	nanometre
nM	nanoMolar
nmol	nanomole
nmoles	nanomoles
$\text{N}_2\text{O}_2$	nitrous oxide
$\text{O}_2$	oxygen
PEA	2-phenylethylamine
pH	hydrogen ion potential
PRP	platelet rich plasma

R	gas constant
rpm	revolutions per minute
SD	standard deviation
$^{\circ}\text{T}$	absolute temperature in degrees Kelvin
Tris	2-amino-2-(hydroxymethyl)propane-1,3 diol
$\mu\text{l}$	microlitre
$\mu\text{M}$	microMolar
VMA	3-methoxy-4-hydroxymandelic acid
$V_{\text{max}}$	maximal reaction velocity
$\text{ZnSO}_4$	zinc sulphate

### ABSTRACT OF THESIS

This thesis describes various measurements made in blood platelets and erythrocytes from patients suffering from affective disorders, and the effect a variety of therapies have on these measures.

No significant differences were detected in either platelet membrane ATPase or adenylyl cyclase specific activity in any of the groups of patients studied, when compared to values obtained in control subjects. However, unipolar depressed and bipolar manic patients were shown to have a reduced erythrocyte membrane  $\text{Na}^+ + \text{K}^+$ -ATPase and  $\text{Ca}^{2+} + \text{Mg}^{2+}$ -ATPase activity, respectively. It is suggested that membrane enzyme changes found in some peripheral cells in patients suffering from affective disorders, are not common to all peripheral cells, and may or may not reflect central nervous system changes. The decreased  $\text{Na}^+ + \text{K}^+$ -ATPase activity in erythrocytes was found to be correlated with a reduction in the number of sodium pump sites. This reduction did not appear to be caused by a change in the phospholipid composition of the membrane.

Neither amitriptyline nor mianserin, *in vivo* or *in vitro*, affected platelet membrane ATPase activity. Lithium, *in vitro*, however, stimulated platelet  $\text{Mg}^{2+}$ -ATPase activity. Both amitriptyline and mianserin therapy, and subsequent recovery, caused erythrocyte membrane  $\text{Na}^+ + \text{K}^+$ -ATPase activity in unipolar depressives to approach control values. *In vitro* neither drug affected the erythrocyte membrane. ECT did not affect the reduced  $\text{Na}^+ + \text{K}^+$ -ATPase activity of erythrocyte membranes in unipolar depressed patients. After a course of treatment a slight increase in  $\text{Ca}^{2+}$ -ATPase activity in erythrocyte membranes was observed. Although it is considered likely

that an observed reduction in erythrocyte membrane  $\text{Na}^+ + \text{K}^+$ -ATPase activity indicates a susceptibility to depression, increases in that  $\text{Na}^+ + \text{K}^+$ -ATPase activity do not seem to be obligatory for clinical improvement to take place.

A reduced  $V_{\text{max}}$  for the 5-HT uptake process into platelets was observed in both unipolar and bipolar depressed patients, while  $V_{\text{max}}$  values were found to be elevated in untreated unipolar well patients. Whole blood 5-HT levels in these patients were found to be normal.

Lithium administration did not appear to affect platelet 5-HT uptake. Mianserin therapy and recovery caused a change towards normal  $V_{\text{max}}$  values in unipolar depressed patients. Amitriptyline therapy and recovery caused reduced  $V_{\text{max}}$  values to decrease further,  $K_m$  values for the process to increase, and whole blood 5-HT levels to fall dramatically. *In vitro*, the two drugs acted in a similar manner, causing a decrease in  $V_{\text{max}}$  and an increase in  $K_m$ . As  $V_{\text{max}}$  values appear to be independent of clinical condition in treated patients, a reduced  $V_{\text{max}}$  for 5-HT uptake into platelets from untreated patients appears to represent at most a predisposition towards depression.

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## 1. THE NATURE OF AFFECTIVE ILLNESS

Affective illness is a term which encompasses a large group of mental disturbances which are characterized by phases of depression and elation (mania), either alone or alternately, with lucid intervals. Depression and elation are not the only symptoms of this disorder. Depression is often associated with insomnia, anorexia, feelings of guilt, self-reproach and suicidal tendencies. Associated with elation may be hyperactivity, flight of ideas and marked pressure of thoughts. In severe manic-depressive illnesses, patients often experience delusions and hallucinations (Clare, 1976).

Psychiatrists have unfortunately failed to produce a classification scheme which adequately covers all facets of the disorder (Lewis, 1938; Kendall, 1978; Van Praag, 1978). This is hardly surprising as the aetiology of the illness remains unknown, the pathology uncertain, and the symptomatology is often the subject of debate.

This controversy has stimulated research along several lines. As examination of brain tissue from living patients suffering from affective illness is not possible, the metabolism of these patients has been investigated using urine, blood and cerebrospinal fluid (CSF) in the hope that some abnormality might be detected which would relate to the cause, or at least the progression, of the illness (Van Praag, 1978). Use has likewise been made of post-mortem brain samples taken from individuals who have committed suicide (Lloyd, Farley, Deck and Horneykiewicz, 1974). Tricyclic antidepressants (Kuhn, 1957, 1958; Kielholz and Battegay, 1958), monoamine oxidase inhibitors (Crane, 1956, 1957; Loomer, Saunders

and Kline, 1957), 'novel' antidepressants (Bridges, 1978), lithium (Goodwin, Murphy, Dunner and Bunney, 1972; Schou, 1973) and electroconvulsive therapy (ECT) (Medical Research Council, 1965) have all been shown to be effective in treating these disorders. Investigations have been made of the effects of these treatments in man and animals in the hope that clues might be found to the nature of the illness. More recently workers have investigated a possible genetic involvement (Mendlewicz and Rainer, 1977; Schlessner, Winokur and Sherman, 1979) and the use of animal models has also been considered (Boissier, Godefroy, Soubrie, Thiebot and Weil-Fugazza, 1976; Porsolt, Le Pichon and Jalfre, 1977).

Many hundreds of investigations have been carried out in the hope of detecting metabolic changes in patients with affective disorders and assessing the effects of various therapies. An examination of the results reveals that there are few unequivocal conclusions to be drawn (see reviews by Coppen, 1967; Baer, Platman and Fieve, 1970b; Davis, 1970; Curzon, 1972; Schildkraut, 1973; Hullin, 1976; Jain, 1976; Moyes and Moyes, 1976; Ridges, 1976; Van Praag, 1978).

However, this has not prevented several hypotheses from being advanced to explain the cause of affective illness.

## 2. HYPOTHESES OF AFFECTIVE ILLNESS

### (a) Monoamine hypotheses

Tricyclic antidepressants and monoamine oxidase inhibitors became available for clinical use in the late fifties. It was noticed that the new, chemically unrelated, drugs had two character-

istics in common. Both were efficacious in the treatment of endogenous depressions and both could act like monoamine agonists, albeit through different mechanisms (Van Praag, 1978).

These observations led several workers to suggest that the therapeutic action of these drugs was related to their ability to potentiate monoamines which, for some unknown reason, were depleted in depression. This suggestion was supported by the fact that reserpine, which at that time was used in high doses to treat hypertension, could produce depression in as many as 15% of patients treated with this drug, presumably by reducing the concentration of monoamines in the brain (Bunney and Davis, 1965).

Two theories were advanced, namely the indoleamine hypothesis (Ashcroft, Crawford, Eccleston, Sharman, MacDougall, Stanton and Binns, 1966; Coppen, Shaw, Herzberg and Maggs, 1967; Lapin and Oxenkrug, 1969) and the catecholamine hypothesis (Bunney and Davis, 1965; Schildkraut, 1965) which related the depressive syndrome to a central 5-hydroxytryptamine (5-HT) or catecholamine deficiency, respectively. Mania, if considered at all, was thought to exist at the other end of the biological continuum of mood, and to occur when there was an 'excess' of monoamines in the central nervous system.

#### (i) Urinary studies

Results of investigations measuring indoleamines, catecholamines and their respective metabolites in urine from patients suffering from affective illness have been used to test these hypotheses.

Studies of urinary 5-hydroxyindoleacetic acid (5-HIAA; the major metabolite of 5-HT in both the CNS and the periphery,

Lovenberg and Engelman, 1971), cannot provide a relevant answer. As only 1 to 2% of the 5-HT in the whole body is found in the brain (Cooper, Bloom and Roth, 1978), changes observed in urinary 5-HIAA cannot possibly reflect CNS changes, and thus cannot be used to test the proposed hypotheses.

When results obtained in these studies are assessed it can be seen that there are no consistent 'whole body' changes either.

The 24-hour excretion of 5-HIAA has been found to be decreased (Pare and Sandler, 1959; Van Praag and Leijnse, 1963 a,b), normal (Cazullo, Mangoni and Mascherpa, 1966) and increased (Tissot, 1962) during depression.

Variations in values obtained may have been due to differences in diet. It is known that a variety of foods, especially fruits, contain 5-HT (Crout and Sjoerdsma, 1959).

In a 'longitudinal' study 5-HIAA excretion was found to be greater in mania than during depressive phases (Ström-Olsen and Weil-Malherbe, 1958). Although patients acted as their own controls in this study, the influence of diet cannot be ruled out.

Tryptamine excretion has also been investigated. This compound is formed from tryptophan by the action of amino acid decarboxylase, and an alteration in its concentration might indicate a reciprocal change in that of 5-HT. However, tryptamine excretion has been observed to be both decreased (Coppen, Shaw, Malleon, Eccleston and Grundy, 1965b) and increased (McNamee, Moody and Naylor, 1972) in depression.

Use has also been made of radioactively-labelled precursors administered to patients, to monitor urinary excretion of radio-

actively labelled metabolites. A study where  $^{14}\text{C}$ -L-tryptophan was administered to 5 severely depressed patients (Coppen, Brooksbank and Eccleston, 1974) failed to reveal any difference in the urinary excretion of 5 -  $\{^{14}\text{C}\}$ HIAA either between pre- and post-therapy values, or between patients and controls. The authors commented in their paper that a marked inter- and intra-individual variation existed. An earlier study, which used unlabelled tryptophan (Cazullo *et al*, 1966) had also revealed that urinary 5 - HIAA excretion was normal.

Three studies did find that the urinary excretion of xanthurenic acid and kynurenine was higher in depressed patients, both basally and after administration of tryptophan, compared to control subjects (Cazullo *et al*, 1966; Rubin, 1967; Curzon, 1969). Only a small amount of tryptophan is used for the synthesis of the indole derivatives 5-HT and tryptamine. A much larger amount is converted via kynurenine and xanthurenic acid to the B-vitamin nicotinic acid, or is utilized in protein synthesis. It is conceivable that in the patients investigated, less tryptophan was available in the peripheral circulation, for the synthesis of 5-HT. However, plasma levels of tryptophan were not measured. It is possible that the changes observed were due to alterations in the renal clearance of kynurenine and xanthurenic acid. It is also possible that the increase observed in kynurenine and xanthurenic acid occurred at the expense of tryptamine and not 5-HT. It is interesting to note in this context that urinary 5 - HIAA excretion was found to be normal in these patients (Cazullo *et al*, 1966).

Urinary levels of noradrenaline (NA), dopamine (DA) and their respective metabolites cannot test the hypotheses stringently, either.

The major metabolites of NA in urine are 3-methoxy-4-hydroxy-mandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) (Goodall and Rosen, 1963). Enzyme studies in animals indicate that aldehyde reductase activity predominates in the brain, while aldehyde dehydrogenase activity predominates in the periphery (Erwin, 1973). This suggests that the aldehyde metabolites of NA would follow a predominantly reductive pathway (to the glycol) in the CNS and an oxidative pathway (to the acid) in peripheral tissues. Several animal studies have provided data which suggests that 25 - 60% of the urinary MHPG originates from the metabolism of NA in the brain (Maas and Landis, 1968; Schanberg, Schildkraut, Breese and Kopin, 1968; Maas, Dekirmenjian, Garver, Redmon and Landis, 1973a). In man, Ebert and Kopin (1975), using  $\{^{14}\text{C}\}$  - DA infusions in patients and in control subjects, have suggested that as much as 60% of urinary MHPG originates from brain pools of NA, while virtually all of the urinary VMA results from metabolism of peripheral NA. However, even if these estimates are accurate, it is not possible to say with certainty that an observed change in MHPG excretion reflects a change in NA metabolism in the CNS.

DA is metabolized principally to 3-methoxy-4-hydroxyphenyl-acetic acid (HVA) and to a lesser extent to 3,4-dihydroxyphenyl-acetic acid (DOPAC) (Goodall and Alton, 1968). However, the proportion of urinary DA metabolites that originate from the brain is uncertain. In patients suffering from Parkinsonism, where a

degeneration of the nigro-striatal pathway (a system using dopamine as neurotransmitter) is known to occur, some workers have found urinary HVA excretion to be reduced, while others have found it to be normal (Rine and Sonninen, 1968; Weil-Malherbe and Van Buren, 1969; Calne, Karoum, Ruthven and Sandler, 1969; Tyce, Muentert and Owen, 1970).

NA and DA are also present in urine. However, because of the possibility of peripheral contributions, measurement of the concentration of these compounds in urine cannot provide a measure of CNS activity.

Studies measuring urinary catecholamine levels suggest that there may be a change in 'whole body' catecholamine metabolism in affective illness.

The excretion of NA and normetanephrine (a methylated NA metabolite formed in the metabolic pathway from NA to VMA) has been found to be lower in patients in a depressive state compared to when they are symptom free (Greenspan, Schildkraut, Gordon, Levy and Durell, 1969; Bunney, Murphy and Goodwin, 1970). Decreased NA and normetanephrine excretion is thought to occur in cases of retarded depressions (Schildkraut, 1975).

These differences may well be due to differences in physical activity. It is known that catecholamine excretion can be influenced by muscular activity (Karki, 1956). So, it is possible that the observed decrease in NA and normetanephrine excretion is related to a reduction in activity of the patients concerned. However, Takahashi, Nagao, Tsuchiya, Takamizawa and Kobayashi (1968a) failed to demonstrate a decrease of activity in depression.



It is interesting to note that in manic syndromes (Greenspan *et al* 1969) and in agitated depressions (Sloane, Hughes and Haust, 1966), conditions which are accompanied by motor hyperactivity, the excretion of both NA and normetanephrine has been found to be raised.

Bunney *et al* (1970) found that NA excretion was raised on the day preceding the manic phase in a number of manic depressed patients with rapid phase alteration. This observation raises the possibility that NA excretion might not be exclusively motor determined.

MHPG excretion has been found to be decreased in depressed patients, and also in agitated depressed patients who have an increased excretion of NA and normetanephrine (Maas, Fawcett and Dekirmenjian, 1968; Greenspan, Schildkraut, Gordon, Baer, Aranoff and Durell, 1970).

Maas, Dekirmenjian and Jones (1973b) found that this reduction occurred in both bipolar and unipolar depressed patients. However, a more recent study, investigating a larger number of patients, found a reduction in MHPG excretion in bipolar patients and an increase in excretion in unipolar patients, when they were compared to control subjects (Goodwin and Beckmann, 1975).

Urinary excretion of MHPG has been found to be higher in the manic phase compared to the depressed phase (Greenspan *et al*, 1970; Bond, Jenner and Sampson, 1972; Jones, Maas, Dekirmenjian and Fawcett, 1973). In two manic-depressive patients, MHPG excretion was seen to increase during the days preceding the manic phase (Bond *et al*, 1972). This observation suggests that the raised

excretion is not due entirely to a change in motor activity. Increases in MHPG excretion have been observed in response to various types of stress (Rubin, Miller, Clark, Poland and Arthur, 1970; Maas, Dekirmenjian and Fawcett, 1971). It is possible that observed changes reflect changes in adrenal medulla activity or in CNS metabolism.

The position with regard to VMA is controversial and does not help in deciding the contribution made by central sources. Campanini, Catalano, Derisio and Mardighian (1970) observed an increase in the excretion of VMA in manic patients. However, Greenspan *et al* (1969) measured normal VMA excretion, even in manic patients with raised NA and normetanephrine excretion.

Values obtained for DA excretion are similar to many obtained for NA and MHPG, i.e. increased in mania and slightly reduced in depression (Messiha, Agallianos and Clower, 1970; Schildkraut, 1975).

Studies of urinary cyclic adenosine monophosphate (cAMP) levels have also been undertaken with a view to gaining some insight into CNS metabolism. An important role has been postulated for cAMP in transmission at monoaminergic synapses (for review see Walton, 1977). It is thought that when a neurotransmitter diffuses across the synaptic cleft, it stimulates adenylate cyclase, situated post-synaptically, which causes an increase in cAMP concentration. This increase has been suggested as being responsible for the subsequent response in the effector cell.

Urinary cAMP excretion has been found to be reduced in depressive and increased in manic phases of affective illness

(Abdullah and Hamadah, 1970; Paul, Ditzion, Pauk and Janowski, 1970; Paul, Cramer and Goodwin, 1971). The most severely depressed patients were shown to have the greatest reduction in cAMP (Paul *et al*, 1970). A reduction has been shown to occur in both endogenous and personal depressions (Sinanan, Keatine, Beckett and Love, 1975).

It is more than likely that these changes in cAMP levels relate to motor status (Berg and Glinsmann, 1970; Eccleston, Loose, Pullar and Sugden, 1970) rather than CNS metabolism.

(ii) Plasma studies

Results of studies measuring plasma levels of monoamine precursors in patients suffering from affective disorders have been used to test the monoamine hypotheses. As with urinary studies, it is difficult to assess whether a change observed in plasma levels of precursor would necessarily reflect a change in monoamine levels in the CNS.

Coppen, Brooksbank and Eccleston (1974) demonstrated a slightly reduced tryptophan tolerance in depression. They measured total tryptophan levels in blood at different intervals after i.v. tryptophan administration. During the first three hours after loading, the tryptophan concentration in depressed patients was slightly higher than in controls. This result could be interpreted as being due to an impaired ability of the patients to metabolize tryptophan, or it could be that in the depressed patients the tryptophan was distributed throughout a smaller volume.

Rees, Alltop and Hullin (1974) investigated two patients with rapidly alternating manic and depressive phases. They found that the total plasma tryptophan concentration was decreased in the depressive phase and increased during the manic phase.

It is important, however, to consider the concentration of free tryptophan (i.e. that which is not bound to plasma proteins) for it is this fraction that can be transported across the blood-brain barrier.

The plasma concentration of free tryptophan in females with a depressive illness (no mania) was found to be reduced compared with that in an age-matched control group (Coppen, Eccleston and Peet, 1973). These workers found that after recovery the free tryptophan concentration increased in these patients, but did not attain normal levels. This might have been due to a decrease in the amount of tryptophan bound to plasma proteins. It is interesting to speculate upon what might have produced the change in binding. There was no diet control in the study. Variations in food intake might have been an important factor. Patients did not receive drugs which are known to alter the degree of tryptophan binding (e.g. salicylates), however other substances are known to interfere with this binding. Free fatty acids (FFA) in plasma can influence protein binding of tryptophan (Coppen *et al*, 1973; Lipsett, Madras, Wurtman and Munro, 1973). This fat fraction responds strongly to psychological stress, so it is quite possible that any change monitored in free tryptophan concentration was a non-specific phenomenon.

It is impossible to determine if 5-HT concentration in the brains of depressed patients are reduced as a consequence of reduced plasma free tryptophan levels. The transport of tryptophan across the blood-brain barrier can be influenced in many ways (see review by Green and Grahame-Smith, 1975).

High levels of circulating hydrocortisone (cortisol) have been found in depression (Hullin, Bailey, McDonald, Dransfield and Milne, 1967). Corticosteroids from the adrenal cortex can stimulate

the synthesis of tryptophan pyrrolase in the liver (Knox and Auerbach, 1955) which is the first enzyme in the conversion of tryptophan via the kynurenine route. These observations tie in with the reported increased urinary excretion of xanthurenic acid and kynurenine in depression.

Rubin (1967) found that the increased kynurenine excretion he observed correlated with an increased plasma cortisol level.

It is likely that activation of tryptophan pyrrolase can withdraw a substantial amount of tryptophan from 5-HT synthesis. Acutely administered hydrocortisone induces in rats an activation of liver pyrrolase, a decrease in plasma tryptophan and a decrease of approximately 30% in cerebral 5-HT and 5-HIAA concentrations (Curzon and Green, 1968). When animals are given a pyrrolase inhibitor in advance, hydrocortisone no longer causes a decrease in intracerebral 5-HT (Green and Curzon, 1968). Another compound,  $\alpha$ -methyltryptophan, which also activates liver pyrrolase but independently of the adrenal cortex, also causes a decrease in central 5-HT concentration (Curzon, 1969). It may be that in depression, as a result of a raised plasma cortisol level, 5-HT concentration in the brain of depressives is reduced. However, it should be noted that Rubin (1967) did not measure plasma tryptophan levels.

Results available from studies of plasma tyrosine levels are equivocal.

In patients with endogenous or vital depressions fasting plasma tyrosine levels were found to be no different from controls (Takahashi, Utena, Machiyama, Kurihama, Otsuka, Nakamura and

Konamura, 1968b). However, when tyrosine was administered orally, plasma tyrosine levels were higher in patients than in controls.

In complete contradiction to this study, Benkert, Renz, Marano and Matussek (1971) found a decreased plasma tyrosine level in depressed patients in the morning hours, but after oral administration of tyrosine, no change in levels was observed.

Prolactin levels (Sachar, Frantz, Altman and Sassin, 1973) and the growth hormone response (Gruen, Sachar, Altman and Sassin, 1975) after L-dopa administration were found to be normal in patients with unipolar and bipolar depressions. The growth hormone response to insulin-induced hypoglycemia, however, was lower in females (all post-menopausal) with unipolar depression than in a control group (Gruen *et al*, 1975). These are interesting observations. The secretion of prolactin by the anterior hypophysis is regulated by dopaminergic neurons of the tuberoinfundibular DA system. Secretion is inhibited by an increase, and stimulated by a decrease in DA activity. This is believed to occur via an increase or decrease, respectively, of the secretion of prolactin-inhibiting factor (PIF), a substance formed by neuroendocrine cells in the hypothalamus (Meites, Lu, Wuttke, Welsch, Nagasawa and Quadrie, 1972). Growth hormone secretion is increased with increased central catecholaminergic activity and decreases with its decrease (Martin, 1973). This regulation is probably effected via an increase and decrease respectively of the secretion of growth hormone releasing factor (GRF) by neuroendocrine cells in the hypothalamus. There are indications that NA (Toivola and Gale, 1972) as well as DA (Müller, Pecile, Felici and Cocchi, 1970) are involved in the regulation of

growth hormone. L-dopa administration (precursor of DA as well as NA) causes a decrease in plasma prolactin and an increase in plasma growth hormone concentrations in humans (Kleinberg, Noel and Frantz, 1971, Kansal, Buse, Talbert and Buse, 1972). The growth hormone level also rises in response to insulin-induced hypoglycemia, which is probably a catecholaminergic effect, for it disappears following administration of reserpine (which depletes monoamine stores) and  $\alpha$ -methyl-p-tyrosine (which inhibits catecholamine synthesis) (Millar, Sawano and Arimura, 1967). Gruen *et al* (1975) findings would seem to suggest that a malfunction in a catecholaminergic system exists in depression. However, no final conclusions can be made until an investigation is made of the peripheral metabolism of prolactin and growth hormone in these patients.

(iii) Platelets and erythrocytes

Several investigators have proposed that the blood platelet can serve as a model for the amine-storing presynaptic nerve terminal in the central nervous system (Sneddon, 1973; Stahl, 1977). Platelets and synaptosomes have many similar characteristics, as detailed in Table 1.1 (see Sneddon, 1973; Stahl, 1977 and Gordon and Olverman, 1978 for references). The proposal that platelets can serve as models of nerve-endings is very attractive in terms of research into affective disorders. Blood samples are easily obtained from patients, and platelets can be readily separated from other blood cells by differential centrifugation. Enzyme activities of, and monoamine uptake into, platelets are measures that have been made in patients with affective illness. However, it is not known



TABLE 1.1: A comparison of platelets and synaptosomes

	Platelet	Synaptosome
Limiting membrane	Yes	Yes
Mitochondria	Yes	Yes
Storage granules	Yes	Yes
Lysosomes	Yes	Yes
Glycogen particles	Yes	Yes
Microtubules	Yes	Yes
Microfilaments	Yes	Yes
Active transport mechanism for:		
Tryptophan	Yes	Yes
5-HT	Yes	Yes
Tyrosine	No	Yes
DA	No	Yes
NA	No	Yes
Adrenaline	No	Yes
Anabolic enzymes present:		
Tryptophan hydroxylase	Disputed	Yes
Aromatic amino acid decarboxylase	Disputed	Yes
Tyrosine hydroxylase	?	Yes
Dopamine- $\beta$ -hydroxylase	No	Yes
Phenyl-ethanolamine-N-methyl transferase	?	Yes
Catabolic enzymes present:		
Monoamine oxidase	Yes	Yes
Aldehyde reductase	Yes	Yes
Aldehyde dehydrogenase	No	Yes
Catechol-O-methyl transferase	No	Yes
Storage of monoamines	Yes	Yes
Release of stored monoamines	Yes	Yes

in the studies which will be described below, how the activities compared with those in the CNS of the patients investigated.

Red blood cells from patients have also been used to estimate possible metabolic disturbances present in affective illness.



These cells, like platelets, are readily obtained. Most investigations using erythrocytes have involved measurement of intracellular ion concentrations and activities of various membrane-bound enzymes (see Section 1, part 2 (c) (iv) of this thesis). It is again not known to what extent these measures reflect those in the brain.

A 50% reduction in monoamine oxidase (MAO) activity was found in platelets taken from bipolar depressed patients (Murphy and Weiss, 1972). The MAO activity remained reduced after recovery from depression and during manic phases. MAO activity in blood platelets is known to be lower in men than in women (Robinson, Davi Nies, Ravaris and Sylwester, 1971). However, this fact does not explain the observations of Murphy and Weiss (1972) as the patients were age and sex-matched to control subjects. In confirmation of their observation, octopamine has been shown to accumulate in blood platelets of depressed patients as a result of reduced MAO activity (Murphy, 1972). However, it is not known if MAO activity in platelets reflects that present in brain. If platelet MAO activity did parallel that of the CNS, it is difficult to reconcile this observation with the monoamine hypotheses, unless one considers it to represent a compensatory change. However, platelet MAO activity has been reported as increased in unipolar depressed patients (Buchsbaum, Landau and Murphy, 1973; Nies, Robinson and Harris, 1974).

Uptake of 5-HT (Hallstrom, Linford Rees, Pare, Trenchard and Turner, 1976; Mulgirigama, 1976; Tuomisto and Tukianen, 1976) and DA (Hallstrom et al, 1976; Mulgirigama, 1976) has been found to be reduced in platelets from depressed patients. Although there is evidence to suggest that 5-HT uptake into platelets may be similar to 5-HT uptake

in the brain (Sneddon, 1973), it is doubtful if the same holds true for DA uptake (Gordon and Olverman, 1977; 1978). The observed reductions in platelet 5-HT uptake, if reflecting the situation in the CNS, could be used to either uphold or refute the serotonergic hypothesis, depending on whether the observed change is considered to be secondary and compensatory in nature, or is considered to be a primary response.

Cohn, Dunner and Axelrod (1970) and Dunner, Cohn, Gershon and Goodwin (1971) measured catechol-O-methyl transferase (COMT) activity in red blood cells in patients with various psychiatric disorders and found a 50% reduction in females with unipolar depression and a slight reduction in bipolar females. No such abnormality was seen in men with either unipolar or bipolar depression or in schizophrenic women. The reduction was shown by these workers not to be syndrome-linked, for when the patients recovered their COMT activities were still reduced. This suggests that the reduced COMT activity may indicate a 'pre-disposition' to depressive illness at most. However, it is not known if this reflects any CNS abnormality. If this observation did parallel a change in the CNS, it is again difficult to reconcile this with the catecholamine hypothesis, unless one considers it to represent a homeostatic change, which occurs as a result of abnormally low levels of catecholamine.

(iv) Blood pressure

Infused NA was found to have a reduced influence on the systolic blood pressure of depressed patients (Prange, McCurdy and Cochrane, 1967). However, it is impossible to infer from this observation whether the metabolism of NA is accelerated or NA receptors are "impaired" in the CNS.

(v) Carbon dioxide

Coppen, Shaw and Malleson (1965a) observed that when  $^{14}\text{C}$ -labelled 5-hydroxytryptophan (5-HTP) is injected i.v. into depressed patients, the production of  $^{14}\text{C}$ -carbon dioxide (in expired air) is decreased when compared to that in a control group. This might suggest a defect in the conversion of 5-HTP to 5-HT, though not necessarily in the brain. However, these workers were unable to reproduce their own results (Coppen, 1967).

(vi) Cerebrospinal fluid

Measurement of levels of indoleamines, catecholamines and their respective metabolites in cerebrospinal fluid (CSF) might be expected to produce values which would more closely reflect those in the brain. Such expectations have prompted many investigations.

In 1960 Ashcroft and Sharman found that the concentration of 'substances with a 5-hydroxyindole structure' in the CSF was reduced in depressive patients. The same laboratory later demonstrated that 5-HIAA was reduced in depressed patients (Ashcroft, Crawford, Eccleston, Sharman, MacDougall, Stanton and Binns, 1966), a finding which was confirmed by several other groups (Denker, Malm, Roos and Werdinius, 1966; van Praag, Korf & Puite, 1970; Coppen, Prange,

Whybrow and Noguera, 1972b; Mendels, Frazer, Fitzgerald, Ramsay and Stokes, 1972a). Some workers, however, reported only very slight decreases in 5-HIAA concentration (Bowers, Heninger and Gerbode, 1969; Papeschi and McClure, 1971) while others found no decrease at all (Roos and Sjöström, 1969; Wilk, Shopsin, Gershon and Suhl, 1972; Sjöström and Roos, 1972; Goodwin and Post, 1973).

These inconsistencies might be explained by differences in the patient groups investigated. A decreased 5-HIAA in lumbar CSF was always found in patients with vital (endogenous) depressions but only occasionally found in patients with personal (reactive) depressions (van Praag, Korf and Puite, 1970). However, the same group could not repeat their own findings (van Praag, Korf and Schut, 1973b). Mendels *et al* (1972a) found that reduced 5-HIAA seemed to be present more consistently in endogenous rather than reactive depressions. They also found a tendency for the reduction to occur more in unipolar than bipolar depressed patients. This was in agreement with the findings of Ashcroft, Blackburn, Eccleston, Glen, Hartley, Kinloch, Lonergan, Murray and Pullar (1973a).

Manic patients have also been shown to have a decreased lumbar 5-HIAA concentration (Denker *et al*, 1966; Bowers *et al*, 1969; Coppen *et al*, 1972b; Mendels *et al*, 1972a). However, normal and increased 5-HIAA CSF concentrations in mania have also been reported (Wilk *et al*, 1972; Goodwin and Post, 1973).

Coppen, Brooksbank and Peet (1972a) found a decreased concentration of tryptophan in lumbar CSF in 10 patients with unspecified depressive syndromes. Ashcroft, Crawford, Cundall, Davidson, Dobson, Dow, Eccleston, Loose and Pullar (1973b) found, however normal levels

of tryptophan in both unipolar and bipolar depressives.

Reduced CSF levels of HVA have been reported in depressed patients (Fapeschi and McClure, 1971). However, many investigations have revealed normal levels (Roos and Sjöström, 1969; Bowers *et al*, 1969; van Praag and Korf, 1971).

In mania reduced CSF levels of HVA have also been observed (Sjöström and Roos, 1972). However, normal values have been reported (Bowers *et al*, 1969; Ashcroft and Glen, 1974).

Reports of CSF levels of MHPG in depression are controversial. Reduced (Post, Kotin, Goodwin and Gordon, 1973) and normal (Wilk, Shopsin, Gershon and Suhl, 1972) values have been observed.

MHPG in mania has been found to be both equal (Post *et al*, 1973) and raised (Wilk *et al*, 1972) compared to a variety of control groups.

It is apparent that the CSF results reported here do not allow any consistent conclusions to be drawn. It is worthwhile considering what may cause the wide variation.

The control groups used in these investigations were not normal subjects, but hospitalized patients with either neurological or psychiatric conditions. Any differences obtained between patients and 'control' subjects must therefore be viewed cautiously.

Differences in the ages of the subjects investigated may cause spurious variations. Bowers and Gerbode (1968a) found 5-HIAA concentration in CSF to increase with increasing age and be significantly higher over, compared with under, the age of sixty years. Ashcroft *et al* (1973a) found a more complicated relationship between age and CSF 5-HIAA concentration, with minimal values in the age range forty to fifty years, and higher values in both younger

and older individuals. Unfortunately, controversy exists here as well, for other groups have found no age correlation at all (Nordin, Ottosson and Roos, 1971; Papeschi and McClure, 1971; Goodwin and Post, 1973).

It is important to consider the motor activity of the subjects investigated, since differences in movement may be associated not only with alterations in amine metabolism itself but also with changes in CSF mixing, thereby causing 'artificial alterations' in CSF metabolites (Post, Kotin, Goodwin and Gordon, 1973). In cases of hyperactivity a raised lumbar CSF 5-HIAA concentration is often found due to more effective mixing of the CSF (Post *et al*, 1973).

In many investigations diet has not been controlled. This could have important consequences. Dietary tryptophan, for example, may produce alterations in brain serotonin turnover (Fernstrom and Wurtman, 1971) and consequently in the levels of 5-HIAA in the CSF of patients with affective disorders.

Differences in diagnostic criteria are other possible sources of inconsistency between studies. In many investigations the nature of the patient populations are not adequately described. Results obtained may vary according to the severity of the illness, whether depressed patients are mainly agitated or retarded, or are unipolar or bipolar .

Investigations after pharmacological manipulation have also been undertaken.

Ashcroft *et al*(1973b) have investigated the CSF concentration of 5-HIAA after tryptophan administration. They found no difference between unipolar and bipolar depressed patients and controls who



had neurological complaints. Another study in the same year investigated changes after tryptophan loading more thoroughly (van Praag, Flentge, Korf, Dols and Schut, 1973a). They measured 5-HIAA and tryptophan levels in CSF at one, two, five and eight hour intervals after tryptophan had been administered to depressed patients. The tryptophan concentration had tripled in depressed patients after one hour, while in non-depressed 'control' patients the values hardly changed. Eight hours after tryptophan administration, the concentration of 5-HIAA in the CSF was much higher in the control group than in the depressed group. These observations led this group to suggest that tryptophan is less easily converted to 5-HT in the CNS of depressed patients.

CSF measurements have also been made in various patient groups after probenecid administration. Probenecid inhibits the transport of several organic acids, including 5-HIAA and HVA, intra-renally (Despopoulos and Weissbach, 1957; Werdinius, 1967). 5-HIAA and HVA are eliminated from the CSF by an active transport process, which is also inhibited by probenecid (Neff, Tozer and Brodie, 1967; Ashcroft, Dow and Moir, 1968). Probenecid administration in test animals is followed by an increase in 5-HIAA and HVA concentration in the brain (Werdinius, 1967) and in the ventricular and cisternal CSF (Guldborg, Ashcroft and Crawford, 1966). The observed increase is a linear one, which lasts for several hours (Bowers and Gerbode, 1968b; Bowers, 1972). This suggests that degradation of amines continues for some time undisturbed, and is not immediately reduced by a feedback mechanism. Probenecid thus allows an estimate of amine turnover to be made.

The transport system for MHPG is relatively unaffected by probenecid; MHPG shows only a slight accumulation in human CSF after administration of the drug (Korf, van Praag and Sebens, 1971; Gordon, Oliver, Goodwin, Chase and Post, 1973). As a result 'NA turnover' cannot be studied.

There may be other shortcomings to the use of probenecid. It is possible that it might interfere with CNS amine metabolism indirectly. Probenecid decreases the serum total tryptophan concentration (Korf, van Praag and Sebens, 1972) and more importantly, increases the plasma free tryptophan concentration (Lewander and Sjöström, 1973). In certain circumstances this could lead to an increase in brain 5-HT levels and therefore to an increase in CSF 5-HIAA concentration (Fernstrom and Wurtman, 1971). Intracerebral tryptophan concentration has been found to increase after probenecid (Korf *et al*, 1972). However, these workers did not observe an increase in 5-HT synthesis. This seems a rather unlikely observation. Synthesis of brain 5-HT is generally considered to be dependant on brain tryptophan levels (see review by Green and Grahame-Smith, 1975). However, work by Barkai, Glusman and Rapport (1972) in cats, which measured the rate of appearance of radioactively labelled 5-HIAA in CSF, confirms that probenecid has no action on 5-HT synthesis.

Probenecid-induced accumulation of 5-HIAA in depressed patients has been shown to be both reduced (Roos and Sjöström, 1969; van Praag, Korf and Puite, 1970) and normal (Goodwin and Post, 1973).

It is possible that inconsistencies are due to differences in the types of depressed patients examined. In 1977 van Praag



found 5-HIAA to be non-significantly lower in depressed patients with a trend toward a bimodal distribution and a low 5-HIAA sub-group. This observation was in general agreement with earlier work by Bowers (1974) who had found 5-HIAA to be high in unipolar but low in bipolar depressed patients.

HVA accumulation in CSF after probenecid has been found to be reduced in patients with endogenous depressions (Roos and Sjöström, 1969; Sjöström and Roos, 1972; van Praag et al, 1973b). This has been considered to reflect the motor retardation seen in this condition (van Praag et al, 1973b). However, normal values have also been reported (Bowers, 1972).

Manic patients have been found to show a decreased accumulation of 5-HIAA (Roos and Sjöström, 1969; Sjöström and Roos, 1972). HVA accumulation in mania has been found to be both normal (Roos and Sjöström, 1969) and reduced (Sjöström and Roos, 1972).

It can be seen that even with the use of probenecid, results are not entirely consistent.

These inconsistencies did not prevent another theory from being presented; namely the modified amine hypothesis (Ashcroft, Eccleston, Murray, Glen, Crawford, Pullar, Shields, Walter, Blackburn, Connechan and Lonergan, 1972; Ashcroft and Glen, 1974). This group considered CSF 5-HIAA measurements made in diagnostic sub-groups of patients with affective illness to be very interesting (Ashcroft et al, 1973a). They found that 5-HIAA was low in recurrent unipolar depression, but normal in bipolar depression and

mania. No change in 5-HIAA was observed following recovery in any of the groups of patients. These workers considered that the low 5-HIAA seen in unipolar depression did not represent a deficiency in the activity of tryptophan-5-hydroxylase in these patients, for when a loading dose of tryptophan was given by mouth, the concentration of 5-HIAA and of tryptophan in the CSF of patients and controls was not significantly different (Ashcroft, Crawford, Cundall, Davidson, Dobson, Dow, Eccleston, Loose and Pullar, 1973b). These findings suggested to them that in unipolar depression there is a change in functional release of 5-HT without a change in the capacity for synthesis. Functional release was not defined by these workers, and presumably does not include the release which occurs after a loading dose of tryptophan. They do not consider alternative explanations for the reduced 5-HIAA seen in unipolar depression i.e. tryptophan deficiency giving rise to a decrease in 5-HT, which perhaps seems more likely given their results after tryptophan administration. These workers feel that in bipolar depression and mania an alternative hypothesis is necessary. Bringing together their observations of exploratory and stereotyped behaviour patterns seen in affective illness and linking them with results from animal studies, they suggest that a change in post-synaptic receptor sensitivity may be the cause of bipolar depression. They appreciate though, that measurements of such post-synaptic changes may be difficult in man.

It is quite possible that fairly gross manipulations

of the CNS, be they pharmacological or physical, may disturb the metabolism of the brain and produce changes in the concentration of metabolites in the cerebrospinal fluid (see van Praag, 1978). However it is not known whether the lesion(s) assumed to exist in the CNS of patients with affective illness involve a small group of cells in the brain or many groups.

When one considers the complexity of the human brain and the mechanisms therein that give rise to the 'mood' of an individual at any one time, it perhaps seems unlikely that differences between so-called normal subjects and manic-depressives (who can be thought of as people who experience 'overshoots' of sadness and happiness) would be revealed from measurements of metabolite concentrations in the CSF.

(vii) Post-mortem brain

Although it might be considered that studies on post-mortem brain could truly test the monoamine hypotheses, such studies are difficult to interpret because of several factors. Post-mortem results can probably be influenced by drugs taken prior to death or in the act of suicide, causes of death in the control subjects, the interval between death and autopsy, age and sex of the subjects involved and the duration of the final agony. In addition, psychiatric ratings of behaviour at the time just preceding death are usually not available.

Shaw, Camps and Eccleston (1967) found that the hindbrain 5-HT concentration was lower in a group of suicide victims than in a control group of individuals who had died as a result of accidents or from acute somatic non-neurologic diseases. They did not measure the concentration of 5-HIAA. Pare, Yeung, Price and Stacey (1969) also found a decreased 5-HT concentration in brains from patients who had committed suicide by coal-gas poisoning, but they reported normal levels for 5-HIAA. Another investigation (Bourne, Bunney, Colburn, Davis, Davis, Shaw and Coppen, 1968) found no differences in 5-HT concentration, but a reduction in 5-HIAA in a suicide group. A more detailed study was undertaken by Lloyd *et al* (1974) who examined discrete areas of the brainstem from suicide victims. A reduction in 5-HT concentration was found exclusively in the raphe nuclei, and that only in the dorsal and the inferior central nucleus. In both those nuclei, 5-HIAA concentration was only slightly lower than in the control group.

Forebrain NA concentration in suicide victims (Bourne *et al*, 1968) was found to be the same as that in controls. Normal levels

of NA in hypothalamus and DA in caudate have been shown (Pare *et al*, 1969).

Gottfries, Oreland and Wiberg (1974) found MAO activity in various areas of the brains of suicide victims to be decreased by between 20 and 40% compared with a control group with a different cause of death. The decrease was observed whether tryptamine or  $\beta$ -phenylethylamine was used as the substrate for the assay. Another study from the same laboratory (Gottfries, Oreland, Wiberg and Wimblad, 1975) measured MAO activity in thirteen different brain areas in fifteen suicides (eight of whom were alcoholics) and twenty control subjects (who had no history of any mental disorder). MAO activity was found to be reduced in the alcoholic suicides compared to the controls. No significant difference was observed, however, between non-alcoholic suicides and controls. Grote, Moses, Robins, Hudgens and Croninger (1974) could not detect abnormalities in either MAO or COMT activity in a study in suicide victims. They found no difference either in dopamine- $\beta$ -hydroxylase activity in these victims compared with controls. It is interesting to note that although brain MAO activity in mice can be influenced by certain states of behaviour (Eleftherion and Boehlke, 1967) it would seem that in brains from presumably very disturbed and depressed people, no change in MAO activity can be convincingly demonstrated.

(b) "System imbalance" hypotheses

Some workers have suggested that depression occurs when both catecholaminergic and serotonergic systems exhibit reduced activity. Mania, on the other hand, has been suggested as occurring when there is increased activity in catecholaminergic systems combined with subnormal serotonergic function (Kety, 1971; Prange, Wilson, Lynn, Alltop, Stikeleather and Raleigh, 1974).

The balance between catecholamine and acetylcholine systems has also been considered. Janowski, El-Yousef, Davis and Sekerke (1972) proposed that certain cholinergic and catecholaminergic systems are reciprocally related and that both could be involved in affective disorders.

They suggested that if cholinergic activity was relatively greater than catecholaminergic, motor retardation would occur concomitantly with a suppressed mood level, and depression would ensue. On the other hand, if catecholaminergic systems were relatively more active than cholinergic, motor activity would be stimulated and mood level would be enhanced, leading eventually to mania. Their suggestions could explain the action of physostigmine in producing motor retardation and lowered mood levels in patients with depressive, manic or mixed schizoaffective syndromes (Janowski, El-Yousef, Davis and Sekerke, 1973; Janowski, El-Yousef and Davis, 1974). Their proposal can be substantiated by the following five observations (see their papers for source references). Reserpine, which can be depressogenic, is a central cholinergic. Tricyclic antidepressants can act as central anticholinergics. Nerve gases and insecticides (which are anticholinesterases) produce mood changes and decrease



motor activity. Methylphenidate, an amphetamine-like drug, increases motor activity and produces a dysphoric or euphoric mood. These effects are abolished by physostigmine. Patients suffering from Parkinson's disease improve when given L-dopa and anticholinergic drugs. It is interesting that these patients often become depressed. This may occur as a result of their drug therapy, which could be producing an imbalance between cholinergic and catecholaminergic systems.

### (c) Electrolyte hypothesis

Another theory of affective illness has centred on the involvement of disturbances in electrolyte metabolism (Coppen, 1967; Baer, Platman and Fieve, 1970b). This hypothesis is not so well defined as the monoamine hypotheses. It suggests that a disturbed sodium metabolism may be principally responsible for the disorder. Interest in this proposal exists largely as a result of the use of lithium in treating affective illness.

### (i) Whole body studies

Depressed patients have been found to retain more sodium than control subjects when 'salt-loaded' (Klein and Nunn, 1945; Klein, 1950). These studies also showed water and sodium balance to fluctuate with mood. A decrease in sodium excretion has been found in normal subjects during 'depressive phases' and an increase in sodium excretion has been shown to occur in manic patients (Schottstaedt, Grace and Wolff, 1956; Ström-Olsen and Weil-Malherbe, 1958). Not all reports agree with these findings. Russell (1960) found no change in total sodium balance. These early studies involved estimating electrolyte concentrations by flame photometry.

The experimental approach to 'balance studies' became more sophisticated with the introduction of tracer and isotope dilution techniques. Using isotopic techniques a decrease in exchangeable sodium has been found in depression (Gibbons, 1960) which did not change when the patients recovered. 'Exchangeable sodium' is the term used to describe the mass of sodium in the body which will mix or 'exchange' with a tracer dose of radioactive sodium. An increasing amount of body sodium exchanges with the isotope over several days;



measurements are usually taken 24 hours after giving the isotope ('24-hour exchangeable sodium') or after full equilibration of the isotope ('total exchangeable sodium'). The lack of effect of recovery was confirmed by a later study but little can be deduced from the results as patients were examined only one day after receiving ECT (Coppen, Shaw and Mangoni, 1962). A more extensive study which included the use of isotopic bromide to estimate extracellular space, found recovery to be associated with an increase in total body water and a decrease in residual sodium (Coppen and Shaw, 1963). Residual sodium was considered to consist of two components, a component of slowly exchanging sodium in bone and sodium in the intracellular compartment of the body. On the basis of these results Coppen and Shaw (1963) suggested that depression was associated with an abnormality in sodium transport which resulted in an increase in the intracellular concentration of sodium, which decreased on recovery. In agreement with these findings, residual sodium has been found to be high in both depressed and manic patients (Coppen, Shaw, Malleison and Costain, 1966; Cox, Pearson and Speight, 1971) with a trend towards a decrease in residual sodium on recovery (Baer, Durell, Bunney, Levy, Murphy, Greenspan and Cardon, 1970a). Although these studies provide an approach to the study of electrolyte distribution in vivo, interpretation of such results is fraught with problems.

Results from studies of exchangeable sodium have provided no consistent pattern (Jenner, Gjessing, Cox, Davies-Jones, Hullin and Hanna, 1967; Baer et al, 1970a; Cox et al, 1971).

Studies of potassium, calcium and magnesium metabolism in affective illness have not provided any consistent findings (Hullin, 1975).

(ii) Blood and cerebrospinal fluid

The transport of isotopic sodium from blood to lumbar cerebrospinal fluid has been investigated in affective illness. The results from such studies have been contradictory.

Coppen (1960) investigated the rate of entry of  $^{24}\text{Na}$  into the cerebrospinal fluid of 5 groups of patients. The control group consisted of twelve patients who were given a spinal anaesthetic for abdominal operations. The other four groups consisted of schizophrenics, and depressed patients placed in one of three categories; depressed and untreated, treated and recovered, and treated and still depressed. Patients suffering from a depressive illness were shown to have a rate of entry of  $^{24}\text{Na}$  almost half that of the other patients. Patients who had recovered had normal rates of entry. Patients who were treated, but failed to recover, had the same entry rates as the patients with untreated depression.

Later studies failed to repeat these findings (Fotherby, Ashcroft, Affleck and Forrest, 1963; Carroll, Steven, Pope and Davies, 1969). However, Fotherby, Ashcroft, Affleck and Forrest (1963) did find reduced sodium transport in four patients who showed severe depression without paranoid symptoms. Baker (1971) found sodium entry into cerebrospinal fluid to be reduced in both manic and depressed patients compared to schizophrenics and other controls.

Combining results from his own investigations and from all previous studies on sodium entry into cerebrospinal fluid,

Carroll (1972) considered that sodium entry into CSF was significantly reduced in manic and depressed patients, and that it increased significantly on recovery.

Fotherby *et al* (1963) also obtained results in a group of patients who were exercised by walking around the wards, with assistance from nurses if necessary, for the period between injection of the  $^{24}\text{Na}$  and the lumbar puncture. This group found that exercise significantly increased the transfer rate of  $^{24}\text{Na}$  and suggested that decreased physical activity in depressed patients could account for the differences in the Na transfer rate.

(iii) Post-mortem brain

Samples of homogenized fore-brain have been analysed for water content, total lipids, deoxyribonucleic acid, nitrogen, sodium, potassium, magnesium and chloride in 19 control subjects who died from acute physical illness or accident, and in 11 depressed subjects who committed suicide (Shaw, Frizel, Camps and White, 1969).

Depressed subjects were observed to have a raised water content and a reduced sodium concentration (expressed as both mEq/100mg wet weight and mEq/100mg water) when compared to controls.

Shaw *et al*(1969) draw together their results with those of other workers who have found normal sodium and potassium concentrations in CSF in depression and assumed that this means that concentrations of sodium and potassium in all brain extra-cellular spaces are also normal. Following on from this they suggest that the findings in depressed subjects are due to abnormalities in the relative sizes of intra- and extracellular compartments and in the composition of the intracellular phase. They then suggest by a most tenuous argument, that in brains from depressed subjects, the extracellular space has shrunk, the potassium concentration in cells is low and the concentration of sodium in cells is raised.

Other uncertainties exist in this study. Psychiatric diagnosis was performed after the death of the subjects. At the end of their study a physician in psychological medicine examined the coroner's records of the subjects who had died by suicide and decided if depression had been experienced.

This study cannot add weight to the electrolyte hypothesis.

(iv) Erythrocytes

Red blood cells have been used to study intracellular electrolyte concentrations and associated transport processes.

Results obtained for sodium have been contradictory. Erythrocyte sodium concentration was reported to be lower in depressed patients than in controls (Naylor, McNamee and Moody, 1970a; Mendels, Frazer and Secunda, 1972b; Glen and Bellinger, 1973) but other studies have failed to confirm this (Naylor, McNamee and Moody, 1971). Naylor and colleagues found erythrocyte sodium concentration to decrease as patients recovered from depression (Naylor, McNamee and Moody, 1971; Naylor, Dick, Dick, Le Poidevin and Whyte, 1973) but this was not confirmed (Mendels, Frazer, Secunda and Stokes, 1971; Mendels et al, 1972b).

Studies of active sodium transport in the erythrocyte showed no difference between depressed patients and controls (Naylor, McNamee and Moody, 1970b). These workers did find that the passive permeability of the erythrocytes from depressed patients was lower than that found in four control subjects. In addition Naylor et al (1970b) found active sodium transport to increase as the patients recovered from depression.

Other workers have found reduced active sodium transport in erythrocytes from manic-depressive patients during the manic phase of the illness (Hokin-Neaverson, Spiegel and Lewis, 1974).

As active sodium transport is considered to be brought about by the activity of  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ , changes in active

sodium transport might come about as a result of altered  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity. Such considerations led to a series of studies of  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in erythrocyte preparations from depressed patients. The results of such studies have shown the ATPase activity to increase as patients recovered from depression (Naylor et al, 1973) as predicted if depression is associated with reduced sodium transport which increases on recovery. Naylor and his co-workers (1973) suggested that depression was associated with a decrease in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity, a decrease in active sodium transport and an increase in intracellular sodium concentration. However, they appreciated that their results did not provide the evidence for such a hypothesis.

In a later unsuccessful attempt to sort out the controversy Naylor, Dick, Dick and Moody (1974) found erythrocyte  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  to increase on recovery from depression with no change in sodium concentration.

Other laboratories have made fairly intensive studies of ATPase activity and electrolyte concentrations in erythrocytes in affective illness (Hesketh, 1976; Hesketh, Glen and Reading, 1977; Hesketh, Loudon, Reading and Glen, 1978). Untreated unipolar depressive patients were shown to have a reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity compared to control subjects (Hesketh, Glen and Reading, 1977) which was found to increase on recovery (Hesketh, Loudon, Reading and Glen, 1978) with a concomitant increase in the erythrocyte sodium concentration. These workers considered

that this apparent anomaly (i.e. increased  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity and increased sodium concentration on recovery) might have been caused by lithium (the drug used to treat the patients) increasing the intracellular sodium concentration by inhibiting active efflux of sodium.

Erythrocyte membrane  $\text{Na}^+ + \text{K}^+ -$  and  $\text{Ca}^{2+} - \text{ATPase}$  activities have been found to be lower in women suffering from endogenous depression compared to age-matched controls (Choi, Taylor and Abrams, 1977). These workers did not measure intra- erythrocyte electrolyte concentrations.

More recent work by Frazer , Mendels, Brunswick and Ramsay (1978) investigated the efflux of  $^{22}\text{Na}$  from erythrocytes in vitro, under experimental conditions which allowed the measurement of efflux due to active transport, passive diffusion and exchange diffusion. Their data, from 7 male bipolar depressives, did not provide evidence either for an abnormality in  $^{22}\text{Na}$  transfer across the red cell membrane or for an effect of the lithium ion on such a transfer. This group did not measure ATPase activity, however.

A recent report (Akagawa, Watanabe and Tsukada, 1980) suggests that an increase in erythrocyte  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity may occur in mania.



(v) Saliva

The sodium content of saliva has been estimated in patients suffering from affective disorders (Glen, Ongley and Robinson, 1968). Using a small-volume microelectrode system measuring sodium concentration as pNa, patients with manic-depressive or recurrent depressive illness were compared with healthy individuals. Salivary flow rate was also monitored. This is an important measure. The final composition of the saliva depends on the rate of flow and the rates of selective reabsorption or secretion of ions across the collecting duct walls of the salivary gland.

A greatly increased sodium concentration (corrected for flow rate) was found in the patient group examined, some of whom were depressed and some of whom were manic. Glen *et al* (1968) concluded that this increased sodium concentration came as a result of diminished sodium transport across the duct walls.

Data presented in a scatter diagram in their paper, suggests that the manic patients were no different from the depressed patients.



(d) Phenylethylamine hypothesis

In 1974 Sabelli and Mosnaim advanced the hypothesis that a deficiency of 2-phenylethylamine (PEA) in the brain could be a factor in the precipitation of depression.

PEA has been found in human brain (Mosnaim, Inwang & Sabelli, 1974) and in human urine (Jepson, Lovenberg and Zaltzman, 1960) in the same concentration as catecholamines and 5-HT. PEA is produced from phenylalanine by aromatic amino acid decarboxylase, and is structurally related to amphetamine. Monoamine oxidase-B has been shown to act on PEA to produce phenylacetaldehyde (Neff and Yang, 1974). It is interesting that tricyclic antidepressants inhibit MAO-B preferentially (Edwards and Burns, 1974; Roth and Gillis, 1974). If it is true that a PEA deficiency is responsible for depression, the action of tricyclic antidepressants could be explained by their ability to retard the metabolism of this substance, thereby causing an increase in its concentration in the brain. Both tricyclics and monoamine oxidase inhibitors have been shown to cause an increase in the intracerebral concentration after administration to rats (Fischer, Spatz, Heller and Reggiani, 1972a; Fischer, Spatz, Saaverdra, Reggiani, Moir and Heller, 1972b; Sabelli and Mosnaim, 1974).

However, although the urinary excretion of PEA has been observed to be reduced in endogenously depressed patients (Fischer, Heller and Miro, 1968; Fischer *et al*, 1972a; Sabelli and Mosnaim, 1974) it is not known if a similar reduction is present in the brains of these patients. It must also be remembered that the physiological role of PEA is unknown, although its structural similarity to amphetamine might suggest that it could act as an 'endogenous stimulant'.

### 3. THE TREATMENT OF AFFECTIVE ILLNESS

#### (a) Tricyclic antidepressants

Imipramine (a dibenzazepine derivative), amitriptyline (a dibenzocycloheptadiene derivative), and other closely related compounds are the drugs currently most widely used in the treatment of depression and are often classified as the tricyclic antidepressants because of their structure.

Although synthesized in the 1940s, it was not until the late 1950s, that the antidepressant action of imipramine was established (Kuhn, 1958).

Several chemically related compounds have since been shown to possess antidepressant activity. In addition to the dibenzazepines, imipramine and its secondary-amine congener (and major metabolite) desipramine, and its 3-chloro analog; clomipramine, there are amitriptyline and its N-methylated product nortriptyline (dibenzocycloheptadienes), as well as doxepin (a dibenzoxepine) and protriptyline (a dibenzocycloheptatriene). There is little evidence to suggest that there are any important differences in the efficacy of these compounds (Gilman, Goodman and Gilman, 1980).

If these drugs are given over a period of time to depressed patients, an elevation of mood occurs. About 2 to 3 weeks must pass before the therapeutic effects of the drug are evident. The explanation of the slow onset of effects is a matter of conjecture.

One might expect an effective antidepressant drug to have a mood-elevating or stimulating effect when given to a normal subject. This does not in fact occur. Often unpleasant anticholinergic effects appear and the subject becomes very anxious (Gilman, Goodman and Gilman, 1980).

Tricyclic antidepressants can potentiate the action of biogenic amines in the CNS by blockade of their major means of physiological inactivation; re-uptake at nerve terminals. However, there is increasing doubt that this is either a necessary or sufficient explanation of the antidepressant action of these drugs. Similar doubts concerning the role of altered amine metabolism in the patho-biochemistry of manic-depressive illness have already been discussed. This reservation concerning the mode of action of antidepressants is supported by the observation that several antidepressants are unable to potentiate the effects of biogenic amines. Moreover, other agents that are potent inhibitors of the transport of NA, notably amphetamine and cocaine, are poor antidepressants, despite the fact that they have stimulant and even euphoric effects in some people.

There are additional interactions between antidepressants and amine neurotransmitters that are less completely understood. These include apparent  $\alpha$ -adrenergic antagonistic actions (U'Pritchard, Greenberg, Sheehan and Snyder, 1978), a gradually increasing release of NA (apparent release in quantum released per nerve impulse) that may be related to pre-synaptic  $\alpha$ -adrenergic autoreceptors (Crews and Smith, 1978), neurophysiologically defined stabilizing effects on NA-containing cells of the locus coeruleus (Svenssen and Usdin, 1978) and a gradually evolving increased sensitivity of forebrain neurons to 5-HT (DeMontigny and Aghajanian, 1978). In addition, desensitization of  $\beta$ -adrenergic systems may occur as a sequel to the prolonged exposure of the synapse to NA, due to the inhibition of uptake, perhaps as a result of reduction of the number of available receptors

(see Sulser and Robinson, 1978). It has yet to be determined that slowly evolving changes in receptor number or sensitivity, or the activity of central noradrenergic systems can help to account for the typically prolonged onset of the clinical effects of anti-depressant drugs.

It has been suggested (van Praag, 1978) that if disorders in central monoamine metabolism play a part in the pathogenesis of depression, then it is to be expected that not all tricyclic compounds will be equally effective i.e. a depressive patient with a presumed central 5-HT deficiency would benefit most from a relatively selective inhibitor of 5-HT uptake, whereas a patient with a suspected NA deficiency would respond better to a compound which chiefly inhibits NA uptake. Maas, Dekirmenjian and Jones (1973b) found that the therapeutic efficacy of imipramine and desipramine (block uptake of NA more than 5-HT) is higher when the pre-therapeutic MHPG excretion in urine is low than when the excretion is normal or increased. The low MHPG excretors responded to d-amphetamine by an improvement of mood, whereas the normal MHPG excretors did not. The patients who improved in response to imipramine, desipramine and d-amphetamine showed a moderate increase in renal MHPG excretion; this was not observed in patients who showed no improvement. However, it is unlikely that renal MHPG excretion is solely indicative of decreased NA degradation in the CNS.

(b) "Novel" antidepressants

Several new drugs have recently been developed which have formulae unrelated to the antidepressant compounds previously available (Bridges, 1978).

One of these drugs, mianserin, has a structure which is dramatically different from the conventional antidepressants.

Mianserin, a tetracyclic piperazino-azepine compound, was first synthesized in 1966 (Van der Burg, Bonta, Delobelle, Ramon and Vargaftig, 1970). It was hoped that it would show anti-5-HT and anti-histamine activity, and thus be potentially useful in treating migraine and asthma. Initial studies, however, gave disappointing results. It was noted, however, that mianserin seemed to have a marked mood elevating effect in some subjects (Peet and Behagel, 1978). The finding that mianserin might be useful in treating depression was surprising as classical animal pharmacological tests failed to predict any anti-depressant activity for the compound (Peet and Behagel, 1978). Mianserin, however, was shown to be effective in the treatment of depressive illness and anxiety states, but ineffective in schizophrenia (Itil, 1975). It was shown to possess a similar efficacy to amitriptyline (Itil, Polvan and Hsu, 1972; Coppen, Gupta, Montgomery, Ghose, Bailey, Burns and de Ridder, 1976; Vogel, Bente, Feder, Helmchen, Müller-Oberlinghausen, Bohacek, Mihovilovic, Bränli, Fleischhauser and Walcher, 1976; Jaskari, Ahlfors, Ginman, Lydecken and Tienari, 1977) and imipramine (Murphy, 1975; Pichot, Dreyfus and Pull, 1978). It has also been demonstrated as being superior to placebo in the treatment of depressive illness in general practice (Murphy, Donald and Molla, 1976) and in a double-

blind trial in depressed female in-patients (Smith, Naylor and Moody, 1978a). However, in one double-blind study of mianserin, imipramine and placebo in depressed subjects (Perry, Fitzsimmons, Shapiro and Irwin, 1978), clinical ratings showed that the three treatment groups improved equivalently during hospitalization. This may have been due to the fact that all three groups received intensive psychotherapy during the trial. It should also be noted that the patients were monitored for only 3 weeks. Drug-placebo differences might well have emerged if the investigation had continued for a longer time.

Mianserin appears to have a rather different pharmacological profile from that of the tricyclic antidepressants. This novel antidepressant, administered *in vivo*, has been shown to increase the turnover of NA in rat brain with no concomitant action on biogenic amine uptake. This action can be compared with that of the tricyclics which tend to reduce the turnover of NA and 5-HT and block uptake (Leonard, 1974; Leonard and Kafoe, 1976; Kafoe, De Ridder and Leonard, 1976). However, mianserin has been shown to rival both imipramine and amitriptyline in its ability to block *in vitro* the noradrenergic neurone membrane amine pump of rabbit brain stem slices (Goodlet, Mireylees and Sugrue, 1977) and rat hypothalamic synaptosomes (Raiteri, Angelini and Bertollini, 1976).

Studies using synaptosomes prepared from rat hypothalamus have shown the ability of mianserin to inhibit 5-HT uptake *in vitro* to be appreciably less than that of the tricyclic antidepressants (Goodlet *et al*, 1977). Studies using synaptosomes prepared from rat striata have revealed that mianserin *in vitro* can stimulate



$^3\text{H}$ -5-HT release whereas, imipramine is ineffective (Raiteri *et al*, 1976). This could be due to either a 'true releasing effect' or an inhibition of reuptake of the  $^3\text{H}$ -5-HT spontaneously released. Raiteri *et al* (1976) consider mianserin to have a 'true releasing' action, as imipramine, the strongest inhibitor of 5-HT uptake, among the drugs they tested, did not stimulate release. However, the design of their uptake experiments is such that they can only measure 'net uptake'. They interpret their results as if 'true uptake' was measured. They also assume that a flow rate of 0.5 ml per minute, which is used in the perfusion chamber in their release studies, will prevent re-uptake. They present no evidence for this assumption. Interpretation of the real differences between the two drugs is further complicated in their experiments. In the uptake studies, synaptosomes were exposed to substrate and test drug simultaneously.

The effect of mianserin on 5-HT uptake into platelets has also been investigated. Mianserin (10 $\mu\text{M}$ ) had no effect on 5-HT uptake into rat platelets (Raiteri *et al*, 1976) whereas the same concentration of imipramine produced an uptake inhibition of 85%. In human platelets, mianserin produced a 50% inhibition of 5-HT uptake at 170 $\mu\text{M}$ , whereas imipramine produced an inhibition of 50% at 28nM (Bhargava, Matthijsen and Schönbaum, 1977). All these investigations measured net uptake (=amount taken up - amount released during experiment).

The pharmacology of other 'novel antidepressants' has been reviewed by Bridges (1978).

### (c) Monoamine oxidase inhibitors

The MAO inhibitors comprise a rather mixed group of drugs that have in common the ability to block oxidative deamination of naturally occurring monoamines. That such compounds might be useful in treating depression was revealed when iproniazid was used in the management of tuberculosis in the 1950s. It was found that iproniazid had mood-elevating effects in tuberculous patients (see Gilman, Goodman and Gilman, 1980). Following investigations by Crane (1956, 1957) and Loomer *et al* (1957) it was applied in psychiatry for the treatment of depressed patients. This use in psychiatry has now become rather limited due to often unpredictable interactions with many other drugs and food-derived amines, and the tendency to damage the hepatic parenchyma (Gilman, Goodman and Gilman, 1980). Thus, MAO inhibitors are used when tricyclic antidepressants give an unsatisfactory result and when ECT is inappropriate or is refused.

The capacity of MAO inhibitors to act as antidepressants has most often been assumed to reflect the increased availability of one or more monoamines in the CNS, although this assumption has been difficult to prove. One problem with this theory is that the acute biochemical and pharmacological actions of MAO inhibitors precede the antidepressant effect by as long as two or more weeks. Reasons for this delay remain unexplained. The situation with regard to inhibition of MAO may be even more complicated because it appears that MAO exists in at least two forms with dissimilar substrate preferences and differential sensitivity to selective inhibitors (see Jain, 1976).



(d) Tryptophan

The enzyme tryptophan hydroxylase is not normally saturated with substrate, and administration of tryptophan to test animals results in increased 5-HT synthesis in the CNS (Fernström and Wurtman, 1971). If a 5-HT deficiency is present in depression, it is rational to suppose that administration of tryptophan may correct the proposed deficiency (Van Praag, 1978).

Tryptophan has been considered to be as effective an antidepressant as ECT and imipramine (Coppen *et al*, 1967, 1972a; Jensen, Fruensgaard, Ahlfors, Pihkanen, Tuomikoski, Ose, Dencker, Lindberg and Nagy, 1975). However, several other groups were unable to substantiate such a claim (Carroll, Mowbray and Davies, 1970; Bunney, Brodie, Murphy and Goodwin, 1971; Dunner and Goodwin, 1972; Dunner and Fieve, 1975; Mendels, Stinnet, Burns and Frazer, 1975).

L-tryptophan has also been administered to manic patients (Prange, Wilson, Lynn, Alltop, Stikeleather and Raleigh, 1974). Surprisingly enough, it was found to be more effective than chlorpromazine. This observation could be used to suggest that a 5-HT deficiency existed in mania. However, the effect could just as well be explained by the sedative-hypnotic action of the drug (Gilman, Goodman and Gilman, 1980).

Administration of tryptophan is possibly not a very efficient method of increasing the central 5-HT concentration. No more than 3% of the tryptophan in the diet is converted to 5-HT, and only a small fraction of this becomes involved in 5-HT production in the CNS. Most of the tryptophan is acted on by

tryptophan pyrrolase in the liver. Some tryptophan is used in protein synthesis and a small quantity is converted to tryptamine. However, in humans, the probenecid-induced accumulation of 5-hydroxyindoleacetic acid (the metabolite of 5-HT) in the lumbar CSF shows a marked increase in response to tryptophan administration (Dunner and Goodwin, 1972). This indicates that 5-HT synthesis can be stimulated in humans. However, whether this reflects synthesis in the brain or in the spinal cord is a matter for some conjecture (see Van Praag, 1978). It is also necessary to consider the possible compartmentation of 5-HT in the brain (see review by Green and Grahame-Smith, 1975). These workers consider it difficult to monitor the functional state of 5-HT dependent neurons, because two species of 5-hydroxyindoleacetic acid may exist, one deriving from released 5-HT and the other from non-released 5-HT oxidatively deaminated in the neuron. One example that Green and Grahame-Smith (1975) site to demonstrate this possible compartmentation is that of behavioral changes produced in rats by administration of either tryptophan alone (no effect), or when combined with a MAO inhibitor (hyperactivity). They suggest that with tryptophan loading alone the increased 5-HT synthesis does not exert functional activity because of the binding capacity of intraneuronal vesicles and the activity of intraneuronal MAO. When this enzyme is inhibited, although 5-HT is synthesized at an equivalent rate, it will presumably saturate storage sites and spill over into the synaptic cleft to exert a post-synaptic action. So, it is possible that when tryptophan is administered to humans, and an increase in 5-hydroxyindoleacetic acid is observed, this may not provide evidence for an increase in functionally active 5-HT.



(e) Lithium

The earliest reference to the use of lithium is probably that by Soranus of Ephesus in the 5th century AD, who advocated alkaline mineral waters for the treatment of manic excitement (Johnson and Cade, 1975). Several spa waters have subsequently been found to contain high levels of lithium. The first rational medicinal use of lithium occurred in the 19th century. Lithium salts were administered to gout-sufferers on account of the high solubility of lithium urate. In retrospect it has been pointed out that in certain cases of gout-associated depression, lithium may have had some beneficial effect on the mental symptoms (Schou, 1957). Lithium bromide was used as an hypnotic and anti-epileptic drug briefly in the 1920s. In 1949 Cade discovered lithium salts had a tranquilizing effect when given to guinea pigs and were useful in the treatment of mania (Cade, 1949). Cade's original observation was followed by a series of clinical trials using lithium carbonate for the treatment of mania and depression. It is now accepted that lithium carbonate is an effective treatment for mania (Coppen, 1973; Schou, 1973; Mendels, 1975; Peet, 1975) and certain cases of depression (Goodwin, Murphy, Dunner and Bunney, 1972; Mendels, Secunda and Dyson, 1972c). Manic depressive disturbances are recurrent in that untreated episodes will remit spontaneously and then after a period of time the patient will relapse (Coppen, 1973). There is evidence that the interval between episodes decreases as the patient suffers successive episodes. Lithium appears to reduce the severity of an episode, the frequency of episodes or abolishes altogether future attacks (Coppen, 1973; Schou, 1973; Schou and Thomsen, 1975).

The general biology and pharmacology of the lithium ion have been reviewed in detail by Schou (1957). Despite extensive research it is not known how lithium alters the mood of patients suffering from affective disorders.

The possibilities being pursued include the action of lithium on the sodium pump, the cellular sodium and potassium concentrations and the membrane potential (Ploeger, 1974; Boardman, Hume, Lamb and Polson, 1975; Hesketh *et al*, 1978). A most important characteristic of the lithium ion is that it has a relatively small gradient of distribution across biological membranes, unlike sodium and potassium; while it can replace sodium in supporting a single action potential in a nerve cell, it is not an adequate substrate for the sodium pump and it cannot therefore, maintain membrane potentials.

Other investigations have concentrated on the interactions of lithium with magnesium or calcium in modulating allosteric protein function (Birch, 1973).

Further work has dealt with the influence of lithium on synaptic transmission and the metabolism of catecholamines and related neurotransmitters. In animal brain tissue, lithium ion concentrations of 1 to 10 mEq per litre inhibit the depolarization-provoked and calcium dependent release of NA and DA, but not 5-HT from nerve terminals (Baldessarini and Lipinski, 1975). It may also slightly alter the re-uptake and presynaptic storage of catecholamines in directions consistent with increased inactivation of the amines. Although this could explain the action of lithium in restraining mania, it is difficult; in terms of the catecholaminergic hypothesis, to thus explain the efficacy of lithium in treating depression. Chronic lithium treatment has been found to decrease

5-HT and GABA binding in rat brain with no change in  $\beta$ -adrenergic or muscarinic binding (Maggi and Enna, 1980). However, an earlier study showed that lithium reduced  $\beta$ -adrenergic receptor binding in rat brain too (Treiser and Kellar, 1979).

Therapeutic concentrations of lithium have almost no discernable psychotropic effects in normal man (Schou, 1957). One must question the relevance of measuring lithium actions on normal animal brains in the hope of finding out how lithium acts in affective illness.

Studies investigating metabolic changes in humans after lithium administration have revealed no consistent trends which could be used to produce a unifying theory of affective disorders, (van Praag, 1978).

(f) Antipsychotic drugs

Antipsychotic drugs have been used with some success in mania and depression. This group of drugs, effective in the treatment of psychoses, include compounds such as the phenothiazines, the structurally similar thioxanthenes, and the dibenzidiazepines and dibenzoxazepines, butyrophenones (phenylbutylpiperidines) and the newer diphenylbutylpiperidines, indolones and other heterocyclic compounds, and the rauwolfia alkaloids and related synthetic heterocyclic amine-depleting agents (Gilman, Goodman and Gilman, 1980).

Haloperidol (a butyrophenone) and chlorpromazine (a representative of the phenothiazine-thioxanthene class) are both effective in the treatment of mania, and are often administered concomitantly with lithium (see Gilman, Goodman and Gilman, 1980). In fact, it is often impractical to attempt to treat a manic patient with lithium alone during the first week of illness, when the antipsychotic drugs are usually required. To date there has been no controlled study of the possible long-term prophylactic effects of antipsychotic drugs in effective illness. The use of phenothiazines for the treatment of depression is more controversial. Some studies have demonstrated the effectiveness of antipsychotic drugs in depressed patients who show marked agitation or psychotic delusions (Overall, Hollister, Meyer, Kimball and Shelton, 1964).

The effects of antipsychotic drugs are apparent at all levels in the nervous system. Theories based on their ability to antagonize the actions of DA as a neurotransmitter in the basal ganglia and limbic portions of the forebrain have become most prominent and are supported by a wealth of data (see Gilman, Goodman and Gilman, 1980 for references).



(g) Electroconvulsive therapy

ECT is a treatment which involves the production of a controlled convulsion. In comparison to other therapies, which require nothing more than the swallowing of a few pills each day, ECT is a more complicated procedure. Atropine is administered to the patient, as a pre-anaesthetic medication, before undergoing ECT, to dry up salivary and bronchial secretions and reduce the likelihood of anaesthetic or stress-induced heart irregularities. The patient is then given an intravenous injection of a short-acting anaesthetic, usually thiopental, and through the same needle, a muscle relaxant, usually succinylcholine. Within fifteen to twenty seconds of the injection, slight rippling of the muscles under the skin can be seen which serves to indicate that muscle paralysis is imminent. At this point the anaesthetist takes over respiration of the patient using a face mask and a pressure bag. When the muscles stop twitching, the shock can be administered. Two electrodes, dampened with a bicarbonate solution to prevent skin burns at their points of contact, are applied to the anterior temporal areas of the scalp (at each side of the head, above and in front of each ear). A gag is then put into the patients mouth to prevent the tongue being bitten. An electric current, usually eightyvolts with a duration of 0.1-0.3 seconds, is given which results in a "modified" convulsion, as shown by the mild tonic-clonic movements in the facial muscles and in the muscles of the hands and feet. After the convulsion, the gag is removed, the patient is turned onto one side, and the anaesthetist maintains an oxygen supply until the muscle relaxant wears off (this occurs within several minutes of it having been given) and

the patient starts to breathe on his own. Within five to twenty minutes the patient gradually returns to full consciousness although still may feel sleepy and indeed may sleep for up to an hour after treatment. The usual course of treatment consists of between five to twelve treatments, given at a rate of two to three per week (Clare, 1976).

The use of electricity in treating depression was described as early as 1792 by John Birch who was a surgeon at St. Thomas's Hospital (Clare, 1976). Birch treated a porter who had been suffering from a melancholic state for almost a year. The treatment Birch gave involved six small shocks which were passed through the brain in different directions on each of three successive days, following which the patient regained his spirits, went back to work and remained perfectly well for seven years.

In more recent times, Cerletti, in 1938, was one of the first to administer electroconvulsive shock to a mentally deranged person; in the form of an incoherent "gibbering catatonic schizophrenic" (Impastato, 1960). Such treatment had been considered acceptable, because of a commonly held belief, at that time, that as schizophrenia and epilepsy were mutually exclusive diseases, the production of a seizure in a schizophrenic might well relieve the disturbed mental state (Meduna, 1938). Such a notion has not stood the test of time.

During the twenty years following that occasion, electroconvulsive therapy gradually became established as the major physical treatment in psychiatric practice. Attention was paid to what constituted the appropriate number and frequency of induced convulsions, the optimal strength and type of current, the most



suitable part of the head to which apply the electrodes, and the psychiatric conditions for which it seemed the most appropriate. The use of a group of drugs capable of briefly paralysing muscles (curare, gallamine, succinylcholine) and of short-acting anaesthetic agents (thiopental, methohexital) meant that ECT could be administered with reasonable safety, few side effects, and a minimum of discomfort. The muscle relaxant ensured that a fit could be induced without a concomitant and massive spasm of the major muscles while the anaesthetic relieved the patient's understandable anxiety during the treatment and much of his post-seizure anxiety as well.

Many physiological and biochemical changes have been recorded in animals after electroconvulsive shock, and in patients suffering from affective illness after ECT.

Grahame-Smith, Green and Costain (1978) have examined the effects of electroconvulsive shock on the behavioural responses of rats to pharmacological stimuli. Results obtained using MAOIs and tryptophan (effects of which involve pre-synaptic mechanisms of synthesis, compartmentation, release and inactivation by re-uptake of 5-HT) and 5-methoxy-N,N-dimethyltryptamine (a putative 5-HT receptor agonist which would produce post-synaptic receptor stimulation) have prompted them to suggest that electroconvulsive shock enhances post-synaptic responses mediated by 5-HT. This could be used as evidence for the serotonergic hypothesis, however using other test systems, electroconvulsive shock enhanced DA and possibly NA post-synaptic responses. Grahame-Smith *et al* (1978) appreciate, however, that these effects are difficult to relate to the therapeutic effect of ECT in depression. In their experimental set-up, electroconvulsive shock is applied to a pres-

umably normal rat brain, the function of which is being disturbed. In man, ECT is applied to a presumably abnormal brain whose function is presumably being returned to normal. A suitable extension of these studies would be an examination of the effects of electroconvulsive shock in an animal model of depression.

Successful ECT has been found to increase CSF HVA and 5-HIAA concentrations in depressed patients compared to schizophrenic patients (Jori, Dolfini, Casati and Argenta, 1975). However, other workers (Abrams, Essman, Taylor and Fink, 1976) found no change in CSF levels of 5-HIAA, tryptophan and HVA after ECT, although all patients investigated showed marked clinical improvement.

Reduced serum magnesium levels in depression have been found to increase to normal values after treatment with ECT (Carney, Sheffield and Sebastian, 1973). However, these workers considered that their results might well have been due to seasonal variation of serum magnesium combined with an unequal distribution of cases throughout the year.

A transient reduction in serum total calcium has been found to occur during a successful course of ECT (Carman, Post, Goodwin and Bunney, 1977). This group did not investigate control subjects. However, a comparison of their baseline and post-ECT values with values reported by other workers (Frizel, Coppen and Marks, 1969) suggests that their calcium levels are in the normal range.

Erythrocyte membrane  $\text{Na}^+ + \text{K}^+ -$  and  $\text{Ca}^{2+} - \text{ATPase}$  activities were investigated by Choi *et al* (1977) in eleven women treated with ECT for endogenous depression and eleven age-matched control subjects. Pretreatment ATPase levels in depressives were significantly lower than in controls, and increased to control levels after a course of ECT.

(h) "Combination" therapy

It might seem rational to combine different antidepressant treatments (i.e. those which would be expected to produce an increase in catecholamine concentration at receptor sites by different mechanisms) if the catecholamine hypothesis were correct, in the hope of producing a more definite anti-depressant response.

Tryptophan (1500mg) and a MAO inhibitor (iproniazid) were administered to twenty endogenously depressed patients. Ten of these patients received the two drugs simultaneously. The remainder received tryptophan after a few weeks of unsuccessful MAO inhibitor treatment. No potentiation in response to the MAO inhibitor was observed (van Praag, 1978). Interpretation of these results is difficult. It is probable that only a small fraction of the tryptophan administered reached the brain.

When larger quantities of tryptophan were administered (214/kg body weight), depressive patients showed a better response to a combination of MAO inhibitor (tranylcypromine) with tryptophan than to a combination of a MAO inhibitor with placebo (Coppen, 1967). It is possible that the type of MAO inhibitor used affects any possible potentiation.

Other drug combinations have been reviewed (see van Praag, 1978). Many combinations (i.e. tricyclic antidepressants + MAO inhibitors and MAO inhibitors + reserpine) although capable of testing the monoamine theory of affective disorders, would never be treatments of choice, because of the possibility of serious cardiovascular side-effects.

#### 4. PROPOSED STUDY

The previous pages have described hypotheses which have been proposed to explain the nature of affective illness, and the experimental data which has been used to test these proposals. Unfortunately the survey is by no means complete. The work discussed reflects the author's interests, as is the case in most reviews of this nature. However, the references quoted cover most aspects of biological research into affective illness.

It is apparent that much uncertainty exists. This uncertainty may in part be due to differences in psychiatric assessment and inter-laboratory variation. It was hoped that by conducting a large scale study in one establishment it would be possible to reduce this variation and thus make more definite statements concerning the nature of the disorder. It was also hoped that additional information would be gained by using a 'finer' psychiatric classification than most studies have used in the past, i.e. one according to the course of the illness (unipolar and bipolar divisions).

It was decided to concentrate on one small aspect of the affective illness controversy and examine blood platelets and erythrocytes from suitable patients. It was outwith the scope of this thesis to test whether changes monitored in blood platelets and erythrocytes reflected similar changes in the CNS of patients with affective illness, where it is presumed the 'precipitating lesion' resides. However, it was hoped that any peripheral changes observed in these patients might be correlated to their changing mood states.

An examination of the effects of various therapies (i.e. lithium, mianserin, amitriptyline and ECT) on these measures was also under-

taken. It would be of great value if there was some means of deciding on the most suitable therapy for a particular subject, by perhaps identifying various subgroups of patients, using some biochemical measure. While patients can be differentiated on the basis of their response to specific therapies, this has the disadvantage that it can only be done retrospectively. It was hoped that by examining blood platelets and erythrocytes from patients when ill and during the course of their recovery, it would be possible to identify such subgroups.

The investigation to be described was designed to bridge the gap between electrolyte, catecholamine and indoleamine studies. Platelet and erythrocyte ATPase activity, platelet adenyl cyclase activity, platelet 5-HT uptake and whole blood 5-HT levels were selected as suitable measures for examination.

It was hoped to test a premise basic to the electrolyte hypothesis. This premise states that a decrease in  $\text{Na}^+ + \text{K}^+$ -ATPase activity, which has been shown to occur in erythrocytes in affective illness, is present in all cells. The activity of  $\text{Na}^+ + \text{K}^+$ -ATPase in platelets was therefore examined.

Mianserin, a drug recently introduced for the treatment of affective disorders, has been classified as a "novel antidepressant". It was hoped that by investigating and comparing it with an archetypal tricyclic antidepressant, i.e. amitriptyline, an estimation could be made of its supposed atypical clinical pharmacology.

It was hoped to obtain some measure of the natural variation which can occur in the various blood platelet and erythrocyte parameters examined. To this end blood samples from a female control subject were studied at intervals over the course of a year.

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## 1. CLINICAL DATA

All patients were referred from within the Royal Edinburgh Hospital which serves a population of 470,000.

### (a) Platelet study

Clinicians withheld active treatment from acutely ill patients until venesection took place, using only a barbiturate for sedation if necessary. Specimens could be processed on any day of the week to avoid unacceptable delay before any patient received specific therapy. Patients who had ingested a tricyclic antidepressant, neuroleptic, or benzodiazepine within two weeks of venesection were excluded. Patients had been diagnosed as suffering from an affective disorder by the referring clinician. They were assessed also by a research psychiatrist using the glossary developed for the Wing Present State examination (Wing, Cooper and Sartorius, 1974) on the basis of the criteria described by Feighner, Robins, Guze, Woodruff, Winokur and Munoz (1972) for affective disorders. A previous episode of mania or hypomania requiring neuroleptic treatment defined a patient as bipolar. Patients receiving lithium carbonate (500 mg - 2000 mg per day) were assessed as to whether they were ill or recovered. A group of normal subjects who received lithium carbonate (500 mg - 1500 mg per day) were not psychologically rated. Hospital and MRC Unit staff were used as a source of control material, and as far as could be ascertained they had no family history of psychiatric disorder nor were they exposed to prescribed drugs.

### (b) Comparative study of platelets and erythrocytes

Clinicians withheld active treatment under the same conditions as in (a). Patients were selected for this study if they were uni-

polar depressed or bipolar manic, and had not ingested a tricyclic antidepressant, neuroleptic, or benzodiazepine within two weeks of venesection. They had been diagnosed by a research psychiatrist as in (a). Students and staff of the MRC Unit and the Department of Pharmacology, Edinburgh University, acted as control subjects. They had no family history of psychiatric disorder nor were they exposed to prescribed drugs.

(c) Mianserin and amitriptyline study

Patients were selected for this study if they had been diagnosed as suffering from unipolar depression by the referring clinician, as in (a), and were to be treated with either mianserin or amitriptyline, and had not ingested a tricyclic antidepressant, neuroleptic or benzodiazepine within two weeks of being admitted to the study. During the investigation patients received either 60 - 100 mg mianserin hydrochloride or 100 - 150 mg amitriptyline hydrochloride per day. Patients receiving mianserin or amitriptyline were assessed as to whether they were ill or recovered. Controls were obtained from the same source as in (b).

(d) Electroconvulsive therapy (ECT) study

Subjects were recruited to the study from patients referred for ECT during a 14-week period in 1978. Study criteria were willingness to take part, depressive illness with no symptoms suggestive of schizophrenia and no ECT in the previous year. All subjects investigated were unipolar depressed patients. Concomitant antidepressant therapy was used for nine patients and this is detailed in the Results Section. ECT was recommended because of poor response to antidepressant drugs, need for rapid recovery or previous good

response to ECT. Changes in clinical state were recorded using the Hamilton Rating Scale (Hamilton, 1960) and reports from each patient's supervising clinician.

Two control groups were used in this study. To examine the effect of anaesthesia without ECT, patients undergoing the relatively atraumatic procedure of diagnostic cystoscopy were selected for investigation. Hospital staff provided another source of control samples (no anaesthetic, no ECT) and as far as could be ascertained they had no family history of psychiatric disorder and had been drug free for at least some weeks prior to sampling.

(e) Arrhenius plots and ouabain binding in erythrocytes

Unipolar depressed patients were selected for this study, as in (a), and control subjects as in (b).

(f) Other studies

In all other experiments described in this thesis blood samples from control subjects were obtained from students and staff of the MRC Unit and the Department of Pharmacology, Edinburgh University. None of these subjects were taking prescribed drugs at the time of venesection and none had a family history of psychiatric illness.

## 2. BLOOD SAMPLING

(a) Platelet study

Patients and controls took only a light breakfast on the morning of sampling but otherwise there was no dietary control. Those receiving lithium took no medication on the morning of sampling. Blood samples were taken from patients receiving lithium therapy, before, 5 days after, 3 weeks after and 3 months after they had started their courses

of medication. Control subjects receiving lithium gave blood before, 5 days after and 3 weeks after starting drug administration.

After applying a venous tourniquet, cubital venous blood was withdrawn through a B - D Yale 20 - g  $1\frac{1}{2}$  microlance into a B - D plasti-pak sterile polypropylene syringe. Nine millilitre units of blood were immediately transferred to the bottom of an all-polythene 10 - ml stoppered tube that contained as anticoagulant 1 ml of 3.8% sodium citrate. The tubes were gently inverted thrice to ensure mixing of the sodium citrate with the blood. The contents of the tubes were then used to prepare platelet rich plasma and platelet membranes.

(b) Comparative study of platelets and erythrocytes

Blood sampling was carried out as in (a). Ten millilitre units of blood were also transferred to the bottom of 10 - ml stoppered sodium heparin blood tubes. The tubes were gently inverted thrice to ensure mixing of the blood with the heparin coating. This blood was used in the preparation of erythrocyte membranes.

(c) Mianserin and amitriptyline study

Blood samples were taken from patients, before, 1 week, 2 weeks and 3 weeks after they had started their courses of medication. Patients receiving mianserin or amitriptyline took no medication on the morning of sampling. Blood sampling was carried out as in (b), the blood being used to prepare platelet rich plasma and platelet membranes, and erythrocyte membranes. Two-and-a-half millilitre units of blood were also transferred into all-polythene tubes containing 0.1 ml (100 i.u.) heparin in deionized water. The tube was gently agitated to ensure mixing of the blood with the heparin. This blood was used for the estimation of whole blood 5 - hydroxytryptamine.

(d) Electroconvulsive therapy (ECT) study

Blood samples were obtained immediately before and ten minutes after the first, second and last ECT. Patients received either unilateral or bilateral ECT under atropine, sodium pentothal and succinylcholine, with ventilation artificially assisted by the anaesthetist. A final sample was taken from fasting subjects one morning twelve weeks after their last ECT. Though 18 patients started in the study, complete samples were obtained from only 4 subjects. This deficiency arose because patients either discontinued ECT (5 subjects), or withdrew their consent (2 subjects), or received a second course of ECT (2 subjects), lithium (1 subject) or died (1 subject) within the twelve-week follow-up period or from whom samples could not be processed on one day because of insufficient material (3 subjects). Consequently, the results refer to 12 patients who were placed in either or both of two groups. Group I comprised nine subjects (A, C, D, H, L, M, N, P, Q) from whom samples were obtained before and after their first, second and last ECT. Group II contained seven subjects (F, H, J, K, N, P, Q) who provided samples before and after their first ECT and at a follow-up twelve weeks later. Details of the patients are given in the Results Section.

Blood samples from the patients undergoing diagnostic cystoscopy were obtained before and about 15 minutes after the investigation, at which time the patients had started to recover from the anaesthetic. The anaesthetic technique used in cystoscopy was different from that used in ECT. Anaesthesia was induced with sodium pentothal and then maintained with  $N_2O_2/O_2$  and halothane. No muscle relaxant was administered.

Blood samples were drawn through a Venflow catheter for the patients undergoing ECT or cystoscopy. Routine venepuncture was used for the other subjects and patients at follow-up. Blood samples were collected in stoppered sodium heparin tubes as in (b). The blood samples were used to prepare erythrocyte membranes.

(e) Arrhenius plots and ouabain binding in erythrocytes

Routine venepuncture was used to obtain blood samples from the subjects in this study. Blood samples were collected in stoppered sodium heparin tubes as in (b). This blood was used to prepare erythrocyte membranes.

(f) Other studies

In all other studies routine venepuncture was used to obtain blood samples from control subjects.

In most experiments, when platelet rich plasma or platelet membranes were to be prepared, blood was anticoagulated with sodium citrate. In one study, however, platelet membranes were prepared from subjects' blood samples which were divided and placed into both 'sodium citrate tubes' and heparin tubes.

Erythrocyte membranes were usually prepared from blood anticoagulated with heparin. In one experiment erythrocyte membranes were prepared from sodium citrated blood.

### 3. PREPARATION OF PLATELET RICH PLASMA

A paper tissue was used to clean the top inner surface of the 10 - ml tubes containing blood. This was to prevent erythrocyte contamination of the plasma under preparation. The tubes were centrifuged using a low speed head in a Mistral 2L centrifuge, for

30 minutes at 800 rpm (125 g) at room temperature (Lingjaerde, 1971) to produce platelet rich plasma. This was used to measure 5-HT uptake into platelets.

#### 4. PREPARATION OF PLATELET MEMBRANES

For any one sample, 4 - ml platelet rich plasma (PRP) were dispensed into a 10 - ml all-polythene tube using an Eppendorf pipette with a polypropylene pipette tip. To this was added 4 - ml of 0.38% sodium citrate. The tube was gently inverted three times. Sodium citrate was added merely to decrease the viscosity of the plasma, thus enabling the platelets to be spun down readily. The tubes were centrifuged using a low-speed head in a MSE Mistral 2L centrifuge at 2000 g for 30 minutes at 4°C. This produced a platelet pellet at the bottom of the tube. The supernatant (platelet - poor plasma) was decanted off and the tubes inverted and allowed to drain for 10 minutes onto a piece of tissue paper. At the end of this time a piece of tissue paper was used to wipe the inner surface of the tube to prevent plasma contamination. Eight millilitres of 0.38% sodium citrate were added to the tubes containing the pellets and the tubes were then whirlymixed for approximately 15 seconds. The tubes were centrifuged as before at 2000 g for 30 minutes at 4°C. The supernatant was decanted off and the tube was allowed to drain as described above. 100 µl 0.1% Triton X100 was added to this platelet rich pellet and a smooth edged glass rod was used to mix. Two millilitres of distilled water were added and the mixture was then whirlymixed for approximately 30 seconds. The tubes were then placed on their sides at -10°C for approximately 30 minutes, or until the mixture had frozen solid. The



tubes were then removed and allowed to thaw at room temperature. They were whirlmixed for 30 seconds and returned to the deep freeze for a further 30 minutes. When they had been removed from the deep freeze and allowed to thaw in a similar manner as before, an aliquot was removed for an assay of adenylyl cyclase. The remaining sample was diluted approximately five times. This solution was used for measuring ATPase specific activities and protein content.

## 5. PREPARATION OF ERYTHROCYTE MEMBRANES

Erythrocyte membranes were prepared using the method described by Hesketh (1976) which is a modified version of the method of Dick, Dick and Tosteson (1969). The blood tubes were centrifuged for 4 minutes at 1000 g on a MSE Mistral 2L centrifuge at room temperature (20°C). The plasma produced was sucked off using an aspirator. The remaining erythrocytes were washed three times in ice-cold isotonic sodium chloride (156 mM) buffered with 5 mM Tris-HCl, pH 7.4. Sufficient NaCl solution was added to give the original volume, ie 10 - ml. Washing was by repeated centrifugation at 1000 g for 4 minutes at room temperature, after mixing. After the third wash the supernatant and the buffy white coat was removed using an aspirator. The washed erythrocytes were resuspended in the cell wash solution to give an approximately 40% haematocrit. 34 - ml 10 mM Tris-HCl buffer, pH 7.4, was added to 6 - ml cell suspension (to achieve haemolysis) in a MSE 70 - ml polycarbonate centrifuge tube. Half the volume of buffer was added, the tube opening covered with parafilm, whirlmixed, and the remaining 17 - ml buffer added, and the tube again covered with parafilm and whirlmixed. The haemolysate was left at 0 - 4°C for 10 to



15 minutes (the tubes were placed in a dish containing ice, and then the dish was placed in a 'cold room'). The outside of the tubes were wiped dry and placed in the 3 x 70 - ml swing-out rotor of the MSE Superspeed 65 centrifuge and centrifuged at 15,000 rpm (28,000 g) for 1½ hours at 10°C. The resultant supernatant was removed with an aspirator, leaving a red membrane pellet at the base of the tube. The membranes were washed three times by resuspension in 10 mM Tris - HCl buffer to 40 - ml, whirlymixed and centrifuged each time at 28,000 g for 1 hour at 10°C. At the end of the final centrifugation a layer of fluffy white or faintly pinkish membranes was present at the bottom of the tube, together with a pellet of cell debris and haemoglobin. The membranes were decanted into a fresh tube for storage, leaving the pellet in the centrifuge tube. This was washed once in 10 mM Tris - HCl (using approximately 3 - ml buffer) and the wash was added to the membranes in the fresh tube. The membranes were whirlymixed and left overnight in a 'cold room' before assaying for ATPase activity.

## 6. PLATELET COUNTING

Platelets were counted in a sodium citrate diluted sample of platelet rich plasma (20 µl PRP added to 1 - ml 0.38% sodium citrate) using a Hawksley standard counting chamber in a phase contrast microscope, at a magnification of 400 times.

## 7. ESTIMATION OF ATPase SPECIFIC ACTIVITY

### (a) Incubation conditions

$\text{Na}^+ + \text{K}^+ - \text{ATPase}$ ,  $\text{Mg}^{2+} - \text{ATPase}$ ,  $\text{Ca}^{2+} + \text{Mg}^{2+} - \text{ATPase}$  and  $\text{Ca}^{2+} -$

ATPase specific activities were measured in the platelet and erythrocyte

membrane preparations according to the method used by Hesketh (1976). The amount of inorganic phosphate liberated during incubation of the membranes with disodium ATP was taken as a measure of ATPase activity. Enzyme activity was measured under three different sets of ionic conditions; in the presence of magnesium as the sole cation, in the presence of calcium and magnesium and in the presence of sodium, potassium and magnesium. The activity in the presence of magnesium alone was defined as the  $Mg^{2+}$ -dependant ATPase activity ( $Mg^{2+}$ -ATPase activity), which was insensitive to the presence of ouabain. The difference between activity in the presence of sodium, potassium and magnesium (defined as total ATPase activity) and activity in the presence of magnesium alone was defined as the  $Na^{+}+K^{+}$ -stimulated  $Mg^{2+}$ -dependant ATPase activity ( $Na^{+}+K^{+}$ -ATPase activity) and this activity could be completely abolished by ouabain. The activity in the presence of both calcium and magnesium was defined as the  $Ca^{2+}+Mg^{2+}$ -dependant ATPase activity ( $Ca^{2+}+Mg^{2+}$ -ATPase activity) and the difference between that and the  $Mg^{2+}$ -dependant activity was defined as the  $Ca^{2+}$ -stimulated,  $Mg^{2+}$ -dependant activity ( $Ca^{2+}$ -ATPase activity). During the routine estimation of ATPases all activities were defined in terms of cation activation without the use of ouabain. The incubation conditions for the reactions are shown in Table II.1. The total volume of the incubation medium was 2.5 - ml. Water was used to make up the volume of the  $Mg^{2+}$ - and the  $Ca^{2+}+Mg^{2+}$ -ATPase incubation media. The tubes containing the incubation media, including the platelet or erythrocyte membrane sample to be assayed, but excluding ATP, were pre-incubated at  $37^{\circ}C$  for 10 minutes. The reaction was started by the addition of ATP and allowed to continue for 30 minutes. Reaction and enzyme blanks were processed with all

assays, water being used instead of the membrane preparation and ATP. All estimations were carried out in triplicate.

In some experiments the *in vitro* action of various drugs on ATPase specific activities in platelet and erythrocyte membranes was examined. The experimental conditions were as described above, with the exception that the membrane preparations were pre-incubated in incubation media which contained the drug to be tested.

TABLE II.1: Incubation conditions for assay of ATPase activities in platelet and erythrocyte membranes.  
The table shows final concentrations (mM) of chemicals used in the incubation media

	Total ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Disodium ATP	3	3	3
MgCl <sub>2</sub>	6	6	6
KCl	5	-	-
NaCl	100	-	-
CaCl <sub>2</sub>	-	-	0.15
Tris - HCl buffer, pH 7.4	30	30	30
Tris - EDTA, pH 7.4	0.1	0.1	0.1

In several experiments, using platelet membranes the concentrations of MgCl<sub>2</sub>, NaCl, KCl and CaCl<sub>2</sub> in the incubation media were altered to see if a change in measured specific ATPase activity resulted. Mg<sup>2+</sup> - ATPase activity was measured in the presence of between 1 mM to 30 mM MgCl<sub>2</sub>, all other constituent concentrations remaining as before. Total ATPase activity (from which Na<sup>+</sup> + K<sup>+</sup> - ATPase activity could be calculated) was measured in the absence or

presence of NaCl (50 mM, 100 mM or 150 mM) and in the absence or presence of KCl (2.5 mM, 5 mM or 7.5 mM), in various combinations as shown in the Results Section, all other constituent concentrations remaining as before.  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity was measured in the presence of between 0.05 mM to 100 mM  $\text{CaCl}_2$ , all other constituent concentrations remaining the same. No attempt was made to correct for the changing osmolarities of the incubation media.

(b) Estimation of inorganic phosphate

Inorganic phosphate was estimated by the method of Stoward as described by Naylor, Dick, Dick, Le Poidevin and Whyte (1973). The ATPase reaction was stopped by placing the tubes in ice and leaving them there for 10 minutes. Each tube was subsequently reacted in turn. 0.5 - ml perchloric acid - tungstosilicic acid reagent (48.5 to 50 - ml perchloric acid; Analar 72%, was added to 300 - ml distilled water, 40 g dodecatungstosilicic acid was added to this and allowed to dissolve and the solution was made up to 500 - ml) and then 1.5 - ml molybdate reagent (14.5 g sodium molybdate dihydrate and 131.3 g sodium chloride were dissolved in 1 litre of distilled water) were added and the sample whirlymixed briefly. Four millilitres ethyl acetate (Analar) was added, the mixture whirlymixed for 30 seconds and the tubes returned to ice. After separation of the two layers, a 3 - ml aliquot of the upper layer was placed into a clean tube. Care was taken, when sampling the upper layer, not to disturb the precipitate of protein at the interface between the layers, lest the aliquots of the organic layer should become contaminated, and prevent an accurate optical density reading being taken. The absorbance at 310 nm was measured in the aliquots of the upper layer in 3 - ml quartz

cuvettes in a Unicam SP 500 Series 2 ultraviolet and visible spectrophotometer. The amount of phosphate in the original samples was calculated by comparison of the optical densities with those from standard phosphate solutions ( $\text{KH}_2\text{PO}_4$  in concentrations of 0.1 mM, 0.05 mM, 0.02 mM, 0.01 mM and 0.005 mM was prepared. Aliquots of 2.5 - ml gave standards of 250 nmoles, 125 nmoles, 50 nmoles, 25 nmoles and 12.5 nmoles of phosphate). Standards were processed with each assay. The ATPase activities are given in this thesis in terms of nmoles inorganic phosphate liberated per hour per milligram of protein (nmol  $\text{P}_i$ /hr/mg protein).

Using this method for estimating inorganic phosphate the ATPase reaction was found to be linear with respect to time (over 1 hour) and amount of membrane preparation added (25 - 200  $\mu\text{g}$  protein). The amount of ATP present at the start of the reaction was well in excess of substrate requirements. At most only 2% of the ATP was hydrolysed, so effects produced by accumulating ADP were minimal.

In view of the high sensitivity of this method sometimes a batch of ATP could not be used because it produced a high blank value.

#### (c) Standard ATPase preparation

Periodically a standard  $\text{Na}^+ + \text{K}^+$  - ATPase preparation was assayed along with the membrane preparation. A sodium iodide extracted fraction of hog cerebral cortex was used. The purchased preparation (from Sigma Chemical Co.) was dissolved in water and aliquots placed in separate tubes. These were freeze-dried and then stored at  $-20^\circ\text{C}$ . When required, tubes were thawed and added to incubation medium.

(d) Erythrocyte  $\text{Na}^+ + \text{K}^+$  - ATPase Arrhenius plots

In one study  $\text{Na}^+ + \text{K}^+$  - ATPase specific activities of erythrocyte membranes were measured at different temperatures to enable Arrhenius plots to be drawn and apparent activation energies to be calculated. It was hoped that these activation energies might reflect the lipid state of the membrane preparation, and that any change in activation energy values would reveal a change in lipid composition (Schwartz, Lindenmayer and Allen, 1975). The  $\text{Na}^+ + \text{K}^+$  - ATPase assay was carried out as described above, with the exception that the assay was performed at temperatures of  $10^\circ$ ,  $15^\circ$ ,  $20^\circ$ ,  $25^\circ$ ,  $30^\circ$ ,  $35^\circ$  and  $37^\circ\text{C}$ . Graphs were constructed for each sample of  $\log_{10}$  ATPase specific activity against  $1/\text{absolute temperature}$  (Dixon and Webb, 1965). The activation energy  $E$  (in units of Kcal/mole) is given by the gradient of this plot which is  $-(\Delta H + RT)/2.303R$ , where  $\Delta H + RT$  is equal to  $E$ , and  $R$  is the gas constant ( $R$  being in units of 1.98 cal per mole per degree).

(e) Effect of change in platelet preparation on platelet membrane ATPase specific activities

ATPase specific activities were measured in some control subjects' platelet membranes which had been prepared in a slightly different manner from that described before. This was to enable an examination of the action that Triton, and freezing and thawing had on the platelet membrane ATPase activity. Table 11.2 shows the various modifications that were made.

TABLE 11.2: Modifications made to platelet membrane preparation  
(all other procedures, as detailed before, remained  
the same)

Triton X100 added	Water added	Freezing - thawing
-	2 - ml	twice
-	2 - ml	once
-	2 - ml	-
100 $\mu$ l	2 - ml	twice
100 $\mu$ l	2 - ml	once
100 $\mu$ l	2 - ml	-
200 $\mu$ l	2 - ml	twice
200 $\mu$ l	2 - ml	once
200 $\mu$ l	2 - ml	-



## 8. ESTIMATION OF ADENYL CYCLASE ACTIVITY

### (a) Incubation conditions

Adenyl cyclase specific activities were measured in platelet membrane preparations. The amount of cyclic adenosine monophosphate (cAMP) liberated during incubation of the membranes with disodium ATP was taken as a measure of adenyl cyclase activity. The incubation medium used in this assay was that of Krishna, Weiss and Brodie (1968), with the exception that unlabelled ATP was used as substrate. The incubation media contained 40 mM Tris - HCl; pH 7.3, 3.3 mM  $MgSO_4$ , 10 mM NaF, 10 mM theophylline, 3 mM  $Na_2ATP$  and an aliquot of the membrane preparation to give a total volume of 0.6 - ml. The Eppendorf tubes containing the incubation media, with the exception of ATP, were pre-incubated at 30°C for 10 minutes. The reaction was started by the addition of ATP and allowed to continue for 10 minutes. Reaction and enzyme blanks were processed with all assays, water being used instead of the membrane preparation and ATP. All estimations were carried out in duplicate. The reaction was stopped by placing the incubation tubes in a heating block at 80°C for 10 mins. The tubes were then spun in an Eppendorf centrifuge for 6 mins. An aliquot of the supernatant was then used to measure the concentration of cyclic AMP present.

(b) Estimation of cAMP

The concentration of cAMP present in the Eppendorf tubes was estimated using cyclic AMP kits obtained from the Radiochemical Centre, Amersham. These kits contain tritium labelled cAMP, a binding protein, an unlabelled cAMP standard solution, Tris/EDTA buffer and a charcoal adsorbent. The assay is based on the competition between unlabelled cAMP, present in the sample to be estimated, and a fixed quantity of the tritium labelled compound for binding to a protein which has a high specificity and affinity for cAMP. None of the incubation medium constituents used in the adenylyl cyclase assay are known to interfere with the binding of cAMP to the protein (Product Information, Radiochemical Centre, Amersham). The amount of labelled protein-cAMP complex formed is inversely related to the amount of unlabelled cAMP present in the assay sample. Separation of the protein-bound cAMP from the unbound nucleotide is achieved by adsorption of the free nucleotide on to coated charcoal, followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. Using known concentrations of unlabelled compound a plot of  $C_0/C_x$  ( $C_0$ : counts bound in absence of unlabelled compound;  $C_x$ : counts bound in presence of standard compound) against the concentration of unlabelled compound can be made. This gives a straight line which enables the concentration of unlabelled cAMP in a sample to be determined. The adenylyl cyclase specific activities are given in units of nmol cAMP produced per 10 minutes per milligram of protein (nmol cAMP/10 mins/mg protein).

Using this method for estimating cAMP the adenylyl cyclase reaction was found to be linear with respect to time (over 20 minutes) and amount of membrane preparation added (150 - 750  $\mu$ g protein).

## 9. MEASUREMENT OF 5-HT UPTAKE

5-HT uptake was measured in aliquots of platelet rich plasma (PRP). Uptake was measured at 37°C and 4°C. The difference between the two values was taken as a measure of active uptake of 5-HT into the platelets. 100 µl PRP was added to 3.9 - ml of pre-warmed (37°C) or ice-cold (4°C) Krebs - Henseleit bicarbonate buffer (pH 7.4), containing  $^3\text{H}$ -5-HT {5-Hydroxy (G- $^3\text{H}$ )} tryptamine creatinine sulphate from the Radiochemical Centre, Amersham, specific activity adjusted to 50 mCi/mmol, and various concentrations of unlabelled 5-HT. Uptake was measured at 5-HT concentrations of 0.25, 0.5, 1.0 and 2.0 µM for each sample. After 5 minutes incubation in a shaking water bath, the uptake at 37°C was stopped by transferring the test-tubes to ice-water. Both sets of test-tubes were centrifuged at 4°C for 30 minutes at 18,000 g using a Mistral 2L centrifuge with a high-speed head. After draining, 1 - ml of distilled water was added to the pellet, and the 5-HT completely released by freezing overnight at -10°C, followed by thawing, and centrifugation at 4°C for 30 minutes at 18,000 g. One-half millilitre of the solution was mixed with 10 - ml NE 260 scintillant fluid and the radioactivity measured in either a Beckman LS 100 liquid scintillation system or a Searle Nuclear Chicago Division mark II liquid scintillation system. The 5-HT uptake was calculated as pmol  $^3\text{H}$ -5-HT/ $2 \times 10^7$  platelets/5 minutes. All estimations were carried out in duplicate. The double - reciprocal plot of Lineweaver and Burke (1934) was used to determine the values of  $K_m$  (Michaelis Menton constant) and  $V_{\max}$  (maximal reaction velocity). This entails plotting  $1/\text{initial velocity of reaction}$  against  $1/\text{substrate concentration}$ . The intercept on the y - axis is  $1/V_{\max}$  and the gradient of the line

is  $K_m/V_{max}$ .  $K_m$  is expressed in units of  $\mu M$  and  $V_{max}$  in units of  $pmol$   $^3H$ -5-HT/ $2 \times 10^7$  platelets/5 minutes.

The purity of the labelled and unlabelled 5-HT was checked using thin layer chromatography. Silica gel plates were used with a butanol - acetic acid - water (5:1:4) mixture as solvent. Formaldehyde was used as the developing agent. This induced a yellow fluorescence in the presence of ultraviolet light (Bartholini and Pletscher, 1964). The presence of one spot only on the plate was taken to indicate that the 5-HT solution did not contain any oxidized 5-HT.

In some experiments the *in vitro* action of various drugs on 5-HT uptake into platelets was examined. The experimental conditions were as described above, with the exception that the platelet preparations were pre-incubated with the drugs for 5 minutes in pre-warmed or pre-cooled Krebs - Henseleit bicarbonate buffer (pH 7.4). The reaction was started by the addition of 5-HT.

#### 10. MEASUREMENT OF WHOLE BLOOD 5-HT

Whole blood 5-HT was estimated according to the method of Ashcroft, Crawford, Binns and MacDougall (1964). One millilitre of heparinized blood was added to 5 - ml distilled water in a graduated 10 - ml glass stoppered centrifuge tube and mixed thoroughly by repeated inversion. The tubes were allowed to stand for 10 minutes to ensure complete lysis. One millilitre of 10%  $ZnSO_4$  was added and the tubes' contents mixed again. 0.1 - ml 20% NaOH was rapidly introduced from an Eppendorf pipette and the solution was mixed immediately. The tubes were centrifuged for 15 minutes at 2000 g on a Mistral 2L centrifuge. Two millilitres concentrated HCl containing 50 mg/100 - ml

ascorbic acid were added to 4 - ml of the supernatant, bringing the solution to approximately 3 N with respect to HCl. Estimation of 5 - HT was then carried out using a Perkin - Elmer Fluorescence Spectrophotometer MPF - 3. An activation scan was made from 250 nm to 400 nm recording at a fluorescence wavelength of 550 nm. A water blank and 5 - HT standard solutions of concentrations of between 10 ng/ml to 200 ng/ml were also processed. All estimations were carried out in duplicate. To check the recovery of this method, 100 ng 5 - HT creatinine sulphate were added to 1 - ml samples of blood. An 80% recovery was found. All samples were corrected for this value.

In one set of experiments the effect of mianserin and amitriptyline on the 5 - HT estimation was tested by processing 5 - HT standard solutions which contained either mianserin HCl in concentrations of 25, 50 or 75 ng/ml, or amitriptyline HCl in concentrations of 500, 1000 or 1500 ng/ml. Neither mianserin nor amitriptyline, in the above concentrations, interfered with the 5 - HT estimation.

#### 11. MEASUREMENT OF OUABAIN BINDING IN ERYTHROCYTE MEMBRANES

Ouabain binding was estimated in erythrocyte membranes in a manner similar to that described by Erdmann and Hasse (1975). Erythrocyte membranes were prepared as usual. After the final centrifugation in their preparation, the membranes, produced from about 20 - ml blood, were decanted into one tube to which 20 - ml 100 mM imidazole - HCl pH 6.5 was added, and were homogenized in this mixture at 4°C. This was then used for the binding assay.

Ouabain binding in the membrane preparations was carried out under two conditions. In one, varying concentrations of <sup>3</sup>H - ouabain,

from the Radiochemical Centre, Amersham, were present (specific activity 15 Ci/mmol, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 120 nM and 160 nM) and in the other 1 mM unlabelled ouabain was present as well (non-specific binding). By subtracting the value obtained for the non-specific binding from that obtained when labelled ouabain was present alone, an estimate of specific binding was made. The incubations were carried out in MSE 5 - ml polycarbonate centrifuge tubes. Each assay tube contained 50 mM imidazole - HCl buffer pH 7.25, 3 mM  $MgCl_2$ , 3 mM imidazole phosphate and either labelled ouabain alone or labelled ouabain and 1 mM unlabelled ouabain. The incubation medium was pre-incubated at 37°C for 10 minutes. The addition of 0.5 - ml membrane suspension, which brought the volume in each tube to 2 - ml, started the reaction. The tubes were incubated at 37°C for 2 hours, previously found to be the time taken for equilibrium to be reached. All estimations were carried out in duplicate. To stop the reaction the tubes were placed on ice and cooled to 0 - 4°C. Following this 2 - ml of a 50 : 50 50 mM imidazole - HCl and 3 mM imidazole phosphate mixture was added to each tube. To the tubes containing only the labelled ouabain 1 mM unlabelled ouabain was added as well. The outside of the tubes were wiped dry and placed in the 6 x 5 - ml swing-out rotor of the MSE Superspeed 65 centrifuge and centrifuged at 28,000 rpm (80,000 g) for 30 minutes at 0°C. The supernatants were decanted and the inside of the centrifuge tubes were wiped dry with a paper tissue. 0.5 - ml of Protosol was added to each tube and allowed to act at room temperature overnight. The samples were transferred to scintillation vials and 10 - ml NE 260 scintillant was added. The radioactivity was counted on a Searle

Nuclear Chicago Division mark II liquid scintillation system.

Scatchard plots were constructed by plotting the ratio of ouabain bound/ouabain free against the concentration of ouabain bound, according to Scatchard (1949). The maximal binding sites can be calculated from the intercept on the ordinate. The gradient of the line gives  $-1/K_D$ , where  $K_D$  is the dissociation constant. The number of binding sites was calculated per mg protein.

## 12. ELECTRON MICROSCOPY

The morphological characteristics of some platelet and platelet membrane preparations were studied by electron microscopy using thin section techniques. Platelet rich pellets, prepared as described before, but to which no Triton had been added and which had not been frozen and thawed repeatedly, were examined. Platelet membranes, prepared as described before, were also examined. When the membrane preparation had been allowed to thaw for the second time, the tubes were centrifuged at  $4^{\circ}\text{C}$  for 30 minutes at 18,000 g using a Mistral 2L centrifuge with a high-speed head. The supernatant was decanted off to reveal a platelet membrane pellet at the base of the tube. The platelet rich and platelet membrane pellets were then both processed in exactly the same way for electron microscopic examination. The pellets were fixed in 1-ml 2.5% gluteraldehyde in 0.5 M sodium cacodylate buffer, pH 7.3. The material was left in fixative for 18 hours at  $0 - 4^{\circ}\text{C}$  and then washed twice in 10% sucrose in cacodylate buffer; each wash was for 10 minutes. Tissue was post-fixed in osmium tetroxide, 1% in cacodylate buffer, for one hour. After dehydrating the tissue in alcohol, 4 ten-minute washes in 10% ethanol



then 4 ten-minute washes in absolute alcohol, the tissue was cleared in epoxypropane (2 ten-minute washes) and then transferred to a thin layer of araldite overnight. This layer was heated at 60°C for 35 minutes and the tissue was transferred to fresh embedding araldite. This was kept at 60°C for a minimum of 3 days. Sections of 500 Å thick were cut through the embedded material using a LKB III ultra microtome and the sections stained in saturated uranyl acetate (15 minutes) and lead citrate (10 minutes). The sections were finally rinsed in 5 mM NaOH and then distilled water. Sections were examined on an AEI EM6M electron microscope using copper grids.

### 13. MEASUREMENT OF PLASMA LITHIUM CONCENTRATIONS

Plasma lithium concentration was estimated by flame atomic absorption at 670 nm using a Perkin-Elmer 360 atomic absorptiometer. A standard LiCl solution of 1 mg/ml, obtained from British Drug Houses Ltd was used to prepare standards of 0.5 mM, 1 mM and 2 mM. Samples and standards were diluted 1:10 for analysis.

### 14. ESTIMATION OF HAEMOGLOBIN

Haemoglobin concentration was estimated in a certain number of preparations, as detailed in the Results Section, according to the method of Bodeman and Passow (1972).

Samples, standards (human haemoglobin, Sigma Chemical Co., 0.1 - 0.5 mg/ml) and blanks were diluted 1 + 4 with glacial acetic acid and left at room temperature for 15 minutes. The absorbance of the solutions was then measured at 398 nm. All estimations were carried out in duplicate.

## 15. ESTIMATION OF PROTEIN

Protein concentration was estimated by a method based on that of Lowry, Rosenbrough, Farr and Randall (1951) following the procedure outlined below:

- (i) Reagents: (A) 2% (w/v)  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH
- (B) 1% (w/v)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
- (C) 2% (w/v) sodium potassium tartrate
- (D) 50 parts of (A) added to 1 part (B) + (C)
- (E) Folin - Ciocalateau reagent diluted 1 + 1.5 with water
- (F) Bovine serum albumin as standard, 15 - 75  $\mu\text{g}$  in 0.3 - ml aliquots

(ii) Procedure: 3 - ml solution (D) was added to 0.3 - ml of samples, standards and blanks (water). After mixing, the solutions were left at room temperature for 15 minutes. 0.3 - ml solution (E) was then added to each tube and the solutions whirlymixed. After standing at room temperature for 30 minutes, the absorbance of the solutions was measured at 750 nm using either a Unicam SP 500 Series 2 ultraviolet and visible spectrophotometer or a Gilford spectrophotometer. All estimations were carried out in duplicate. Protein content of the samples was calculated by comparison of absorbances with those of standard solutions.

## 16. LIQUID SCINTILLATION COUNTING

Liquid scintillation counting efficiency was routinely estimated by counting standard solutions of the radioactively labelled compounds used. Both  $^3\text{H}$  - 5 - HT {5 - Hydroxy (G -  $^3\text{H}$ )} tryptamine creatinine sulphate and  $^3\text{H}$  - ouabain solutions were counted with an efficiency of 25 - 30 per cent.

Both 5-HT uptake and ouabain binding were calculated using dpm (disintegrations per minute) values. In the cAMP estimation, results were calculated using counts per minute as recommended by the Radiochemical Centre, Amersham (Product Information).

## 17. CHEMICALS

All chemicals used were of Analar grade and most were obtained from either British Drug Houses Ltd or Sigma Chemical Co. Heparin BP (mucous) was obtained from Evans Medical Ltd. Mianserin hydrochloride was obtained from Organon Labs. Ltd. Amitriptyline hydrochloride was obtained from Allen and Hanburys Ltd. Protosol was obtained from New England Nuclear, and NE 260 Scintillant fluid from Nuclear Enterprises Ltd.

## 18. STATISTICAL ANALYSES

All results in this thesis are given as means  $\pm$  standard deviation (SD). When comparisons were made, Snedecor's  $f$  value was evaluated first. In quite a large number of the comparisons the  $f$  value was large enough to indicate that a  $t$  - test could not be performed. It was decided therefore to analyse all the data reported in this thesis with non-parametric statistics. Comparisons were made using either a Wilcoxon test for two samples or one for pair differences.

SECTION III

RESULTS

## 1. PLATELET STUDY

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## 1. PLATELET STUDY

(a) Platelet membrane ATPase and adenylyl cyclase specific activities, 5-HT uptake into platelets and platelet counts were measured in control subjects and in untreated patients suffering from an affective illness. The groups of subjects investigated are shown, together with their age and sex, in Table III.1.

No significant differences between untreated patients with affective disorders and controls were found in either platelet membrane ATPase (Table III.2) or adenylyl cyclase (Table III.3) specific activities.  $\text{Ca}^{2+}$ -ATPase activity could not be detected in the platelet membrane preparations. When the results for each group were subdivided into male and female, no apparent differences emerged within each group for either of the parameters measured.

Table III.4 shows  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from controls and untreated patients. There is no difference in  $K_m$  values between the groups studied. A significant reduction in  $V_{max}$  was noted in both the unipolar and the bipolar depressed group.  $V_{max}$  was found to be significantly elevated in the unipolar well group. Classification of the results in Table III.4, divided according to sex, showed no obvious difference between males and females within each group.

No significant differences were found between the groups in platelet counts (Table III.5), and no sex difference in counts in any of the groups could be seen.

TABLE III.1 : Group, age and sex of participating controls and untreated patients

Group	Age	Sex
Controls (26)	37 $\pm$ 10	18F 8M
Unipolar depressed (13)	49 $\pm$ 18	8F 5M
Unipolar well (8)	43 $\pm$ 11	2F 6M
Bipolar depressed (25)	40 $\pm$ 30	16F 9M
Bipolar manic (8)	43 $\pm$ 8	6F 2M
Bipolar well (8)	46 $\pm$ 17	5F 3M

Values are expressed as means  $\pm$  SD.  
Numbers in parentheses refer to the number of subjects in each group.  
Comparisons made with control values by Wilcoxon test for two samples.

TABLE III.2 : ATPase specific activities of platelet membranes from control subjects and untreated patients

Group	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Controls (26)	139 ± 18	742 ± 36
Unipolar depressed (13)	122 ± 20	691 ± 80
Unipolar well (8)	110 ± 50	713 ± 60
Bipolar depressed (25)	148 ± 26	696 ± 92
Bipolar manic (8)	126 ± 49	704 ± 81
Bipolar well (8)	160 ± 63	710 ± 96

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

Numbers in parentheses refer to the number of subjects in each group.

Comparisons made with control values by Wilcoxon test for two samples.

TABLE III.3 : Adenyl cyclase specific activities of platelet membranes from control subjects and untreated patients

Group	Adenyl cyclase activity
Controls (26)	0.20 $\pm$ 0.05
Unipolar depressed (13)	0.19 $\pm$ 0.04
Unipolar well (8)	0.18 $\pm$ 0.34
Bipolar depressed (25)	0.17 $\pm$ 0.05
Bipolar manic (8)	0.19 $\pm$ 0.18
Bipolar well (8)	0.19 $\pm$ 0.11

Activities are expressed as nmol cAMP/10 mins/mg protein.

Values given are means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects in each group.

Comparisons made with control values by Wilcoxon test for two samples.

TABLE III.4 :  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from control subjects and untreated patients

Group	$K_m$	$V_{max}$
Controls (26)	$0.43 \pm 0.05$	$84 \pm 10$
Unipolar depressed (13)	$0.44 \pm 0.07$	$73 \pm 14^*$
Unipolar well (8)	$0.53 \pm 0.23$	$98 \pm 14^*$
Bipolar depressed (25)	$0.46 \pm 0.10$	$59 \pm 35^*$
Bipolar manic (8)	$0.46 \pm 0.06$	$81 \pm 8$
Bipolar well (8)	$0.48 \pm 0.11$	$87 \pm 25$

Activities are expressed as  $\mu M$  for  $K_m$  values and as  $pmol$  5-HT/ $2 \times 10^7$  platelets/5 mins for  $V_{max}$  values. Values given are means  $\pm$  SD.

Comparisons made with control values by Wilcoxon test for two samples.

\*  $P < 0.02$

Numbers in parentheses refer to the number of subjects in each group.

TABLE III.5 : Platelet counts in control subjects and untreated patients

Group	Platelet count
Controls (26)	273 $\pm$ 21
Unipolar depressed (13)	322 $\pm$ 19
Unipolar well (8)	309 $\pm$ 16
Bipolar depressed (25)	329 $\pm$ 25
Bipolar manic (8)	261 $\pm$ 22
Bipolar well (8)	272 $\pm$ 15

Platelet counts are expressed as  $10^3$  platelets/mm<sup>3</sup> blood.

Values given are means  $\pm$  SD.

Comparisons made with control values by Wilcoxon test for two samples.

Platelet membrane ATPase and adenylyl cyclase specific activities and 5-HT uptake into platelets were also examined in several groups of subjects who were receiving lithium carbonate. All results reported here are from subjects whose plasma lithium concentration lay between 0.6 and 1.4 mEq/l.

(b) Tables III.6, III.7 and III.8 show platelet membrane ATPase specific activities, platelet membrane adenylyl cyclase specific activities and platelet 5-HT uptake, respectively, in preparations obtained from 7 control subjects (4 female, 3 male), age  $32 \pm 5$  years, voluntarily taking lithium carbonate. Lithium administration did not significantly affect any of the platelet parameters studied.

A small group of unipolar depressed, bipolar depressed and bipolar manic patients were studied at intervals throughout lithium therapy. Some of the patients examined were prescribed other drugs during their course of lithium. Results given here are from patients who were receiving lithium alone. After 3 weeks of lithium treatment, all the patients, in the groups examined, were classified as unipolar and bipolar well, ie they had recovered while receiving lithium therapy.

(c) Table III.9 gives details of the unipolar depressed patients investigated. Tables III.10, III.11 and III.12 show platelet membrane ATPase specific activity, platelet membrane adenylyl cyclase specific activities and platelet 5-HT uptake respectively, in preparations obtained from these unipolar depressed patients. Lithium therapy and the recovery process appeared not to affect any of these platelet measures.



TABLE III.6 : ATPase specific activities of platelet membranes from control subjects taking lithium carbonate

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Prelithium (7)	124 ± 35	758 ± 45
5 days on lithium (7)	137 ± 22	750 ± 27
3 weeks on lithium (7)	104 ± 38	724 ± 46

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
 Values given are means ± SD.  
 Numbers in parentheses refer to the number of subjects at each stage.  
 Comparisons made with prelithium values by Wilcoxon test for pair differences.

TABLE III.7 : Adenyl cyclase specific activities of platelet membranes from control subjects taking lithium carbonate

Stage of treatment	Adenyl cyclase activity
Prelithium (7)	$0.19 \pm 0.05$
5 days on lithium (7)	$0.19 \pm 0.10$
3 weeks on lithium (7)	$0.18 \pm 0.16$

Activities are expressed as nmol of cAMP/10 mins/  
mg protein.

Values are given as means  $\pm$  SD.

Numbers in parentheses refer to the number of  
subjects at each stage.

Comparisons made with prelithium values by Wilcoxon  
test for pair differences.

TABLE III.8 :  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from control subjects taking lithium carbonate

Stage of treatment	$K_m$	$V_{max}$
Prelithium (7)	$0.45 \pm 0.05$	$85 \pm 5$
5 days on lithium (7)	$0.40 \pm 0.05$	$85 \pm 5$
3 weeks on lithium (7)	$0.46 \pm 0.05$	$82 \pm 13$

Activities are expressed as  $\mu M$  for  $K_m$  values and as pmol 5-HT/ $2 \times 10^7$  platelets/5 mins for  $V_{max}$  values. Values given are means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences.

TABLE III.9 : Unipolar depressed patients treated with lithium carbonate

Stage of treatment	Age	Sex
Prelithium (6)	52 $\pm$ 16	3F 3M
5 days on lithium (6)	52 $\pm$ 16	3F 3M
3 weeks on lithium (3)	41 $\pm$ 11	2F 1M
3 months on lithium (2)	35	2F

Values are expressed as means  $\pm$  SD or mean alone.  
Numbers in parentheses refer to the number of subjects at each stage.  
Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days on lithium' values) and by Wilcoxon test for two samples (for the other values).

TABLE III.10: ATPase specific activities of platelet membranes from unipolar depressed patients taking lithium carbonate

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Prelithium (6)	107 ± 40	702 ± 43
5 days on lithium (6)	126 ± 38	721 ± 67
3 weeks on lithium (3)	101 ± 42	658 ± 33
3 months on lithium (2)	96	660

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values are given as means ± SD or mean alone.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days on lithium' values) and by Wilcoxon test for two samples (for the other values).

TABLE III.11: Adenyl cyclase specific activities of platelet membranes from unipolar depressed patients taking lithium carbonate

Stage of treatment	Adenyl cyclase activity
Prelithium (6)	$0.17 \pm 0.02$
5 days on lithium (6)	$0.18 \pm 0.04$
3 weeks on lithium (3)	$0.16 \pm 0.02$
3 months on lithium (2)	0.17

Activities are expressed as nmol of cAMP/10 mins/mg protein.

Values are given as means  $\pm$  SD. or mean alone.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days on lithium' values) and by Wilcoxon test for two samples (for the other values).

TABLE III.12:  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from unipolar depressed patients taking lithium carbonate

Stage of treatment	$K_m$	$V_{max}$
Prelithium (6)	$0.47 \pm 0.10$	$75 \pm 15$
5 days on lithium (6)	$0.41 \pm 0.07$	$68 \pm 29$
3 weeks on lithium (3)	$0.40 \pm 0.20$	$82 \pm 17$
3 months on lithium (2)	0.42	81

Activities are expressed as  $\mu M$  for  $K_m$  values and as pmol 5-HT/ $2 \times 10^7$  platelets/5 mins for  $V_{max}$  values. Values given are means  $\pm$  SD or mean alone.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days on lithium' values) and by Wilcoxon test for two samples (for the other values).



(d) Details of the bipolar depressed group studied are given in Table III.13. Platelet membrane ATPase specific activity, platelet membrane adenylyl cyclase specific activities and platelet 5-HT uptake are shown in Tables III.14, III.15 and III.16. None of the platelet values in this group seemed to be affected by lithium therapy and recovery.

(e) Table III.17 gives details of the bipolar manic patients who were studied. Tables III.18, III.19 and III.20 show the values obtained for platelet membrane ATPase specific activity, platelet membrane adenylyl cyclase and 5-HT uptake into platelets for this group. The platelet parameters studied were not significantly altered by therapy and recovery from illness.

(f) The *in vitro* action of lithium carbonate on platelet membrane ATPase specific activities and 5-HT uptake into platelets was investigated in platelets from 5 control subjects (1 female, 4 male), age  $29 \pm 12$  years. Table III.21 shows the action of lithium on platelet membrane ATPase specific activities.  $\text{Ca}^{2+}$ -ATPase activity could not be detected in either the presence or absence of lithium. Lithium did not affect  $\text{Na}^{+} + \text{K}^{+}$ -ATPase activity, but did cause a stimulation of  $\text{Mg}^{2+}$ -ATPase activity when present in a concentration of 1 mM or 2 mM. Table III.22 shows the effect of lithium on  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets. No significant change was noted.

TABLE III.13: Bipolar depressed patients treated with lithium carbonate

Stage of treatment	Age	Sex
Prelithium (4)	43 $\pm$ 16	3F 1M
5 days on lithium (4)	43 $\pm$ 16	3F 1M
3 weeks on lithium (4)	43 $\pm$ 16	3F 1M
3 months on lithium (3)	36 $\pm$ 9	2F 1M

Values are expressed as means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparison made by Wilcoxon test for two samples.

TABLE III.14: ATPase specific activities of platelet membranes from bipolar depressed patients taking lithium carbonate

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Prelithium (4)	131 ± 32	700 ± 60
5 days on lithium (4)	146 ± 40	694 ± 36
3 weeks on lithium (4)	129 ± 53	734 ± 42
3 months on lithium (3)	132 ± 43	743 ± 39

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values are given as means ± SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).

TABLE III.15: Adenyl cyclase specific activities of platelet membranes from bipolar depressed patients taking lithium carbonate

Stage of treatment	Adenyl cyclase activity
Prelithium (4)	$0.20 \pm 0.02$
5 days on lithium (4)	$0.18 \pm 0.04$
3 weeks on lithium (4)	$0.19 \pm 0.04$
3 months on lithium (3)	$0.18 \pm 0.05$

Activities are expressed as nmol of cAMP/10 mins/mg protein.

Values are given as means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).

TABLE III.16:  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from bipolar depressed patients taking lithium carbonate

Stage of treatment	$K_m$	$V_{max}$
Prelithium (4)	$0.44 \pm 0.06$	$69 \pm 20$
5 days on lithium (4)	$0.48 \pm 0.14$	$70 \pm 16$
3 weeks on lithium (4)	$0.43 \pm 0.04$	$84 \pm 24$
3 months on lithium (3)	$0.43 \pm 0.09$	$80 \pm 10$

Activities are expressed as  $\mu M$  for  $K_m$  values and as  $pmol\ 5-HT/2 \times 10^7\ platelets/5\ mins$  for  $V_{max}$  values. Values given are means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).

TABLE III.17: Bipolar manic patients treated with lithium carbonate

Stage of treatment	Age	Sex
Prelithium (5)	44 $\pm$ 12	4F 1M
5 days on lithium (5)	44 $\pm$ 12	4F 1M
3 weeks on lithium (5)	44 $\pm$ 12	4F 1M
3 months on lithium (4)	43 $\pm$ 14	4F

Values are expressed as means  $\pm$  SD.  
Numbers in parentheses refer to the number of subjects at each stage.  
Comparison made by Wilcoxon test for two samples.

TABLE III.18: ATPase specific activities of platelet membranes from bipolar manic patients taking lithium carbonate

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Prelithium (5)	114 ± 27	733 ± 63
5 days on lithium (5)	81 ± 42	673 ± 76
3 weeks on lithium (5)	116 ± 35	649 ± 80
3 months on lithium (4)	123 ± 64	697 ± 44

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values are given as means ± SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).



TABLE III.19: Adenyl cyclase specific activities of platelet membranes from bipolar manic patients taking lithium carbonate

Stage of treatment	Adenyl cyclase activity
Prelithium (5)	$0.20 \pm 0.07$
5 days on lithium (5)	$0.17 \pm 0.13$
3 weeks on lithium (5)	$0.18 \pm 0.02$
3 months on lithium (4)	$0.19 \pm 0.10$

Activities are expressed as nmol of cAMP/10 mins/mg protein.

Values are given as means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).

TABLE III.20:  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from bipolar manic patients taking lithium carbonate

Stage of treatment	$K_m$	$V_{max}$
Prelithium (5)	$0.47 \pm 0.09$	$70 \pm 22$
5 days on lithium (5)	$0.43 \pm 0.18$	$72 \pm 22$
3 weeks on lithium (5)	$0.40 \pm 0.16$	$76 \pm 16$
3 months on lithium (4)	$0.48 \pm 0.06$	$70 \pm 22$

Activities are expressed as  $\mu M$  for  $K_m$  values and as  $pmol\ 5-HT/2 \times 10^7\ platelets/5\ mins$  for  $V_{max}$  values. Values given are means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects at each stage.

Comparisons made with prelithium values by Wilcoxon test for pair differences (for '5 days' and '3 weeks on lithium' values) and by Wilcoxon test for two samples (for '3 months on lithium' values).

TABLE III.21: In vitro action of lithium carbonate on ATPase specific activities of platelet membranes from 5 control subjects (1F, 4M)

Concentration of lithium present (mM)	Na <sup>+</sup> + K <sup>+</sup> -ATPase activity	Mg <sup>2+</sup> -ATPase activity
Control	132 ± 29	759 ± 30
0.5	125 ± 19	769 ± 28
1	141 ± 33	780 ± 25*
2	139 ± 21	840 ± 19‡

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made by Wilcoxon test for pair differences.

\* P < 0.02    ‡ P < 0.01

TABLE III.22: In vitro action of lithium carbonate on  $K_m$  and  $V_{max}$  values for 5-HT uptake into platelets from 5 control subjects (1F, 4M)

Concentration of lithium present (mM)	$K_m$	$V_{max}$
Control	$0.46 \pm 0.04$	$85 \pm 12$
0.5	$0.45 \pm 0.07$	$80 \pm 14$
1	$0.43 \pm 0.08$	$83 \pm 13$
2	$0.42 \pm 0.08$	$81 \pm 16$

Activities are expressed as  $\mu M$  for  $K_m$  values and as pmol 5-HT/ $2 \times 10^7$  platelets/5 mins for  $V_{max}$  values.

Values are given as means  $\pm$  SD.

Comparisons made with control values by Wilcoxon test for pair differences.

## 2. COMPARATIVE STUDY OF PLATELETS AND ERYTHROCYTES

ATPase specific activities of platelet and erythrocyte membranes were compared in control subjects, untreated depressed patients and untreated bipolar manic patients. Details of the groups investigated are shown in Table III.23.

(a) ATPase specific activities of platelet membranes from the three groups are shown in Table III.24.  $\text{Ca}^{2+}$ -ATPase specific activity was undetectable. Platelet membrane  $\text{Na}^{+} + \text{K}^{+}$ -ATPase and  $\text{Mg}^{2+}$ -ATPase specific activities are not significantly different in either group when compared with control values.

(b) There is however, a significant decrease in  $\text{Na}^{+} + \text{K}^{+}$ -ATPase specific activity in the erythrocyte membranes of the unipolar depressed group (Table III.25). A decrease in  $\text{Ca}^{2+} + \text{Mg}^{2+}$  ATPase activity in erythrocyte membranes of the bipolar manic group was also observed.

When the results were subdivided into male and female for each group, differences could not be explained on a sex basis.

TABLE III.23: Group, age and sex of participating controls and untreated patients

Group	Age	Sex
Controls (15)	42 $\pm$ 11	7F 8M
Unipolar depressed (12)	45 $\pm$ 12	8F 4M
Bipolar manic (10)	48 $\pm$ 18	6F 4M

Values are expressed as mean  $\pm$  SD.  
Numbers in parentheses refer to the number of subjects in each group.  
Comparisons with control values by Wilcoxon test for two samples.

TABLE III.24: ATPase specific activities of platelet membranes from control subjects and untreated patients

Group	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Controls (15)	163 ± 50	763 ± 38
Unipolar depressed (12)	146 ± 43	730 ± 89
Bipolar manic (10)	132 ± 38	756 ± 93

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

Numbers in parentheses refer to the number of subjects in each group.

Comparisons made with control values by Wilcoxon test for two samples.

TABLE III.25: ATPase specific activities of erythrocyte membranes from control subjects and untreated patients

Group	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Controls (15)	342 ± 49	297 ± 61	870 ± 72
Unipolar depressed (12)	216 ± 36*	317 ± 54	936 ± 80
Bipolar manic (10)	296 ± 60	330 ± 73	726 ± 93*

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made with control values by Wilcoxon test for two samples.

\* P < 0.01

Numbers in parentheses refer to the number of subjects in each group.



### 3. MIANSERIN AND AMITRIPTYLINE STUDY

The action of either mianserin or amitriptyline on whole blood 5-HT and platelet 5-HT uptake was investigated in unipolar depressed patients receiving these drugs. After three weeks drug therapy most patients could be classified as unipolar well. The *in vitro* action of these drugs on platelet 5-HT uptake into platelets from control subjects was also investigated. The groups of subjects investigated, and the estimations performed, are shown in Table III.26, together with their age and sex. The second control group (age  $29 \pm 13$ ) was significantly younger than the other groups.

(a) Figure III.1 shows values obtained for whole blood 5-HT content in untreated control subjects and patients treated with mianserin. Whole blood 5-HT content in untreated patients, before commencing mianserin therapy, was not significantly different from that in controls. Mianserin therapy and the recovery process did not significantly alter the whole blood 5-HT levels.

Whole blood 5-HT content in untreated control subjects and patients treated with amitriptyline is shown in Figure III.2. Values obtained in untreated patients, before commencing amitriptyline therapy, did not significantly differ from that in controls. After one week of amitriptyline therapy, the 5-HT levels in the treated patients were significantly reduced compared to control values but not significantly different from the patients' pre-therapy values (Wilcoxon test for pair differences). After two weeks of amitriptyline therapy the values were reduced even further, to a value significantly

TABLE III.26: Group, age and sex of participating controls and patients

Drug therapy group	Estimations performed	Age	Sex
Controls (10)	Whole blood 5-HT Platelet 5-HT uptake	$42 \pm 25$	5F 5M
Controls (10)	<i>In vitro</i> action of amitriptyline and mianserin on platelet 5-HT uptake	$29 \pm 13$	5F 5M
Mianserin (16)	Whole blood 5-HT	$57 \pm 16$	10F 6M
Mianserin (10)	Platelet 5-HT uptake	$53 \pm 11$	8F 2M
Amitriptyline (8)	Whole blood 5-HT Platelet 5-HT uptake	$43 \pm 17$	5F 3M

Values are expressed as means  $\pm$  SD.

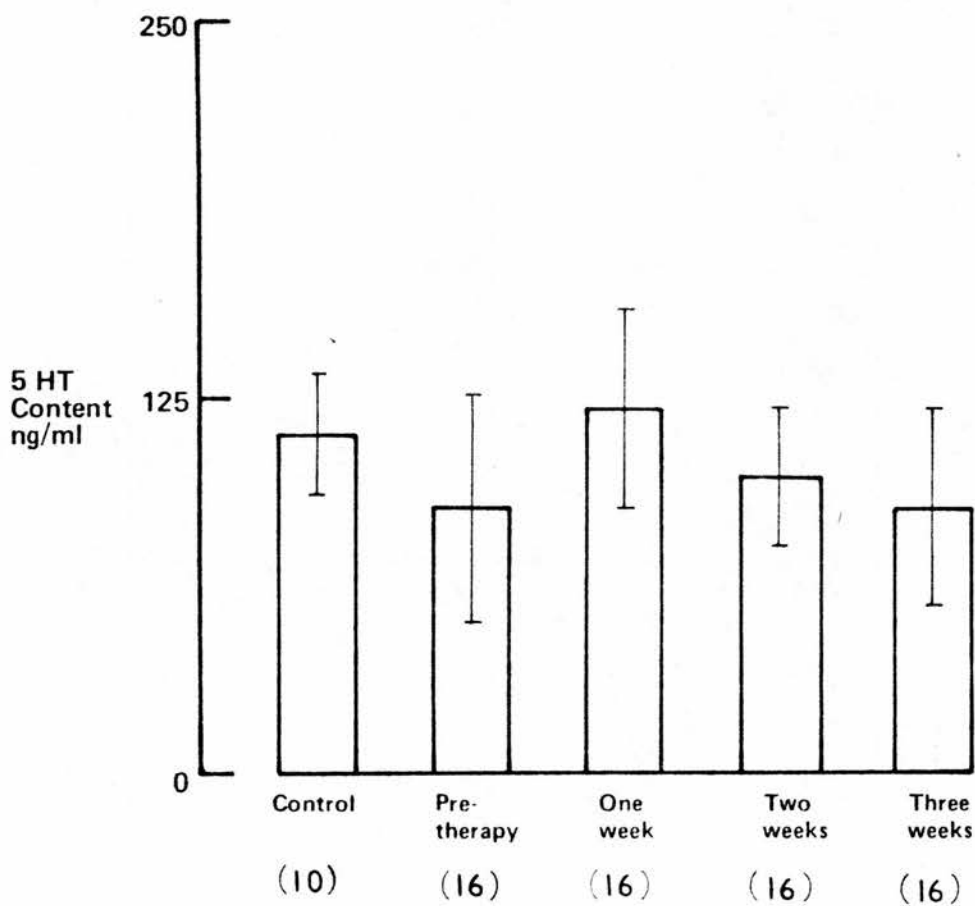
Numbers in parentheses refer to the number of subjects in each group.

Comparison of second control group with first control group by Wilcoxon test for two samples  $P < 0.02$ .

Comparison of second control group with the mianserin groups by Wilcoxon test for two samples  $P < 0.01$ .

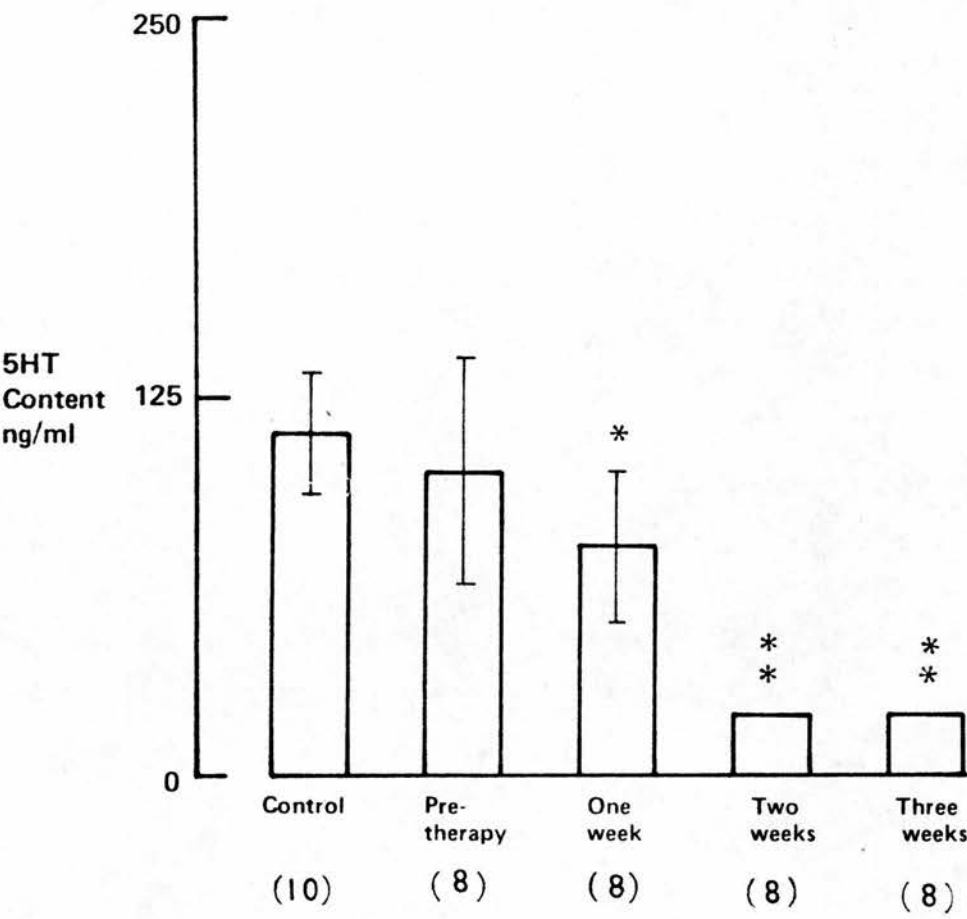
Comparison of second control group with the amitriptyline group by Wilcoxon test for two samples  $P < 0.02$ .

FIGURE III.1 : Whole blood 5-HT content in untreated control subjects and unipolar depressed patients receiving mianserin



Values are expressed as means  $\pm$  SD.  
Numbers in parentheses refer to the number of subjects in each group.

FIGURE III.2: Whole blood 5-HT content in untreated control subjects and unipolar depressed patients receiving amitriptyline



Values are expressed as means  $\pm$  SD.  
Comparisons made with controls by Wilcoxon test for two samples.  
\*  $P < 0.02$     \*\*  $P < 0.01$   
Numbers in parentheses refer to the number of subjects in each group.

lower than either the control value or the pre-therapy value. The levels obtained here were at the limits of sensitivity for the detection of 5-HT by this fluorescence technique, therefore standard deviations are not quoted.

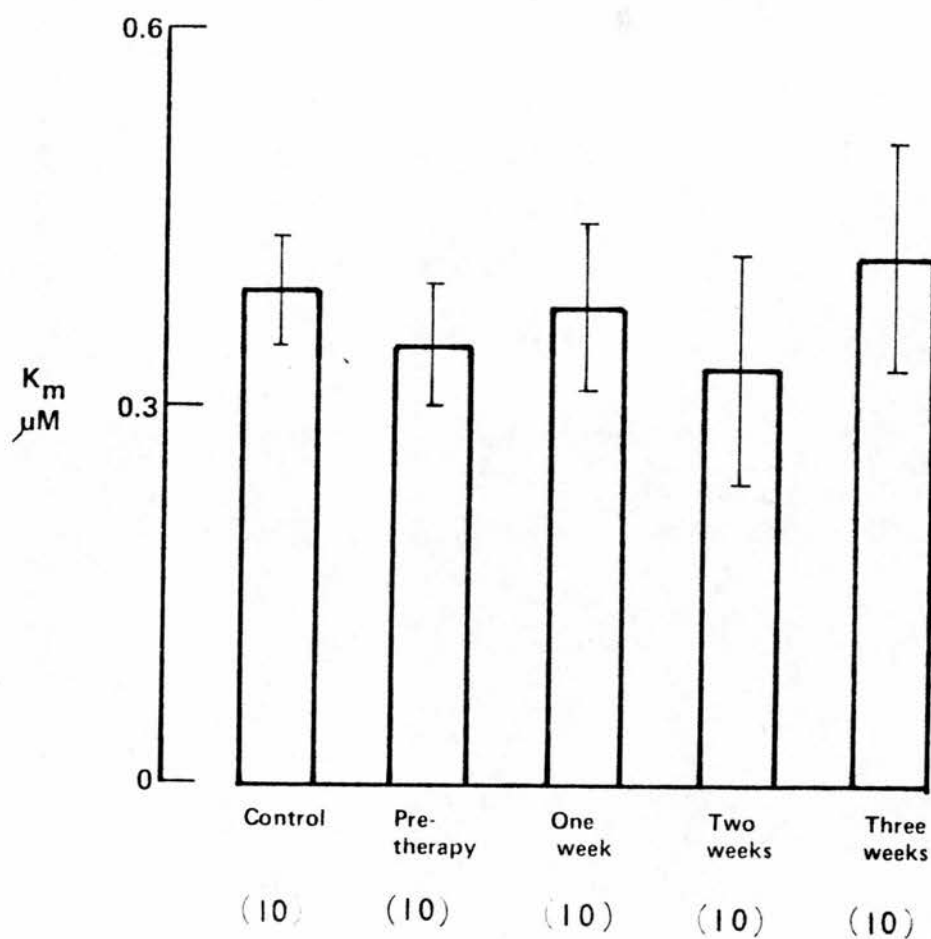
(b) Figure III.3 shows  $K_m$  values for 5-HT uptake into platelets from untreated control subjects and unipolar depressed patients receiving mianserin. The pre-therapy  $K_m$  value was not significantly different from the control value, and the  $K_m$  value was not affected when the patients were treated with mianserin and recovered.

Treatment with amitriptyline for two weeks or more, however, caused an increase in  $K_m$  values (Figure III.4). No difference in  $K_m$  values was found in the untreated patients when compared with controls.

$V_{max}$  values for 5-HT uptake from patients treated with mianserin are shown in Figure III.5. A significant reduction was found in values from untreated patients when compared with controls. Over the course of treatment  $V_{max}$  values tend to increase. After 3 weeks therapy, when the patients had recovered, the values were not significantly different from those of controls, but were significantly different from pre-therapy values ( $P < 0.01$  Wilcoxon test for pair differences).

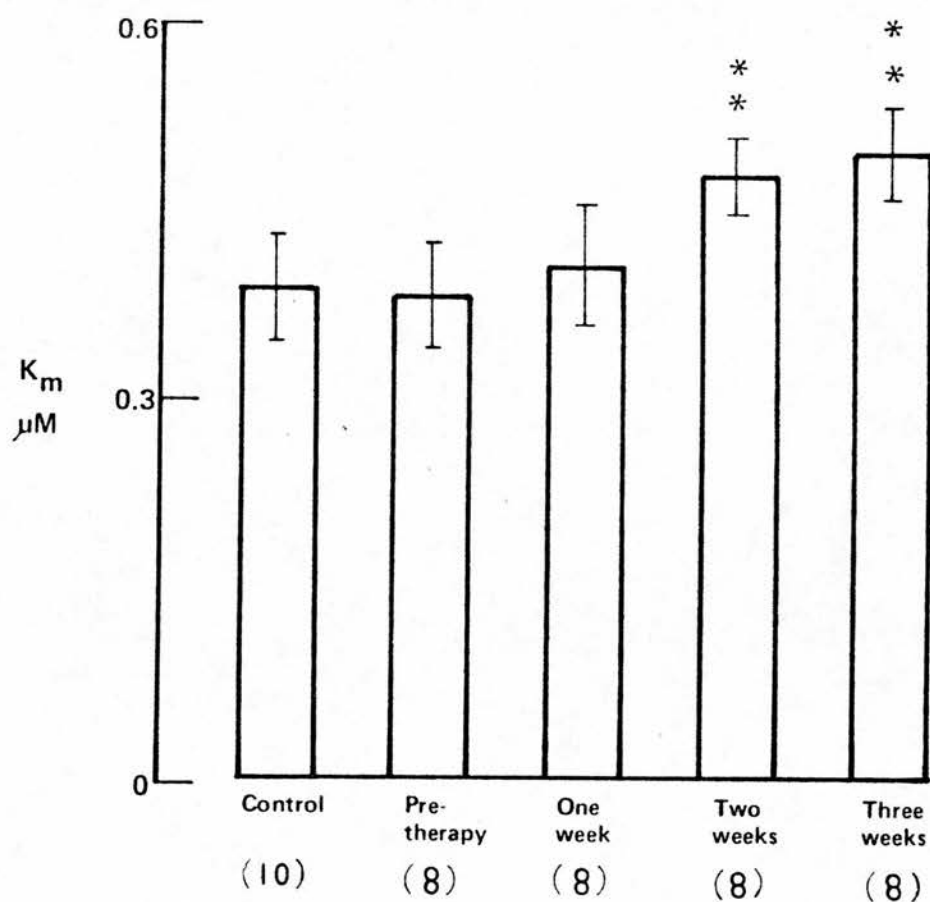
In the unipolar depressed group receiving amitriptyline, pre-therapy  $V_{max}$  values are significantly less than control values (Figure III.6). Treatment with amitriptyline, however, can be seen to cause a further reduction in the value of  $V_{max}$ . After two weeks of amitriptyline therapy,  $V_{max}$  is significantly less than that found before therapy started (Wilcoxon test for pair differences,  $P < 0.01$ ).

FIGURE III.3:  $K_m$  values for 5-HT uptake into platelets from untreated control subjects and unipolar depressed patients receiving mianserin



Values are expressed as means  $\pm$  SD.  
Numbers in parentheses refer to the number of subjects in each group.

FIGURE III.4:  $K_m$  values for 5-HT uptake into platelets from untreated control subjects and unipolar depressed patients receiving amitriptyline

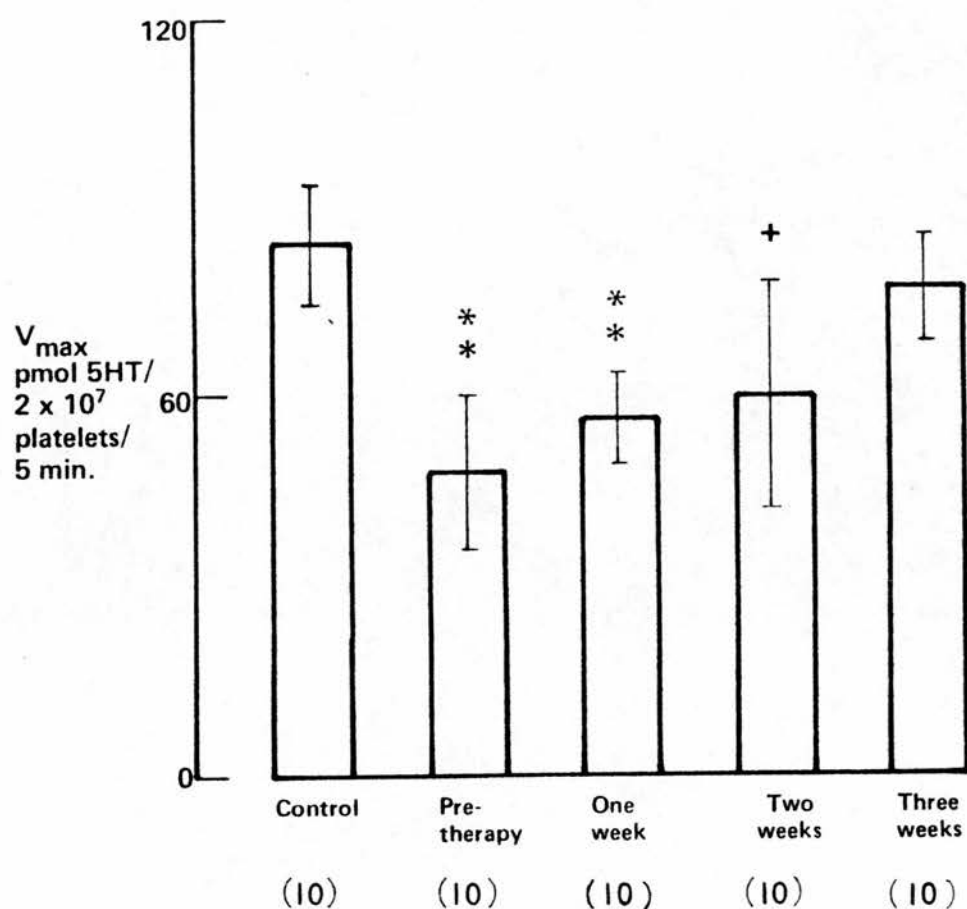


Values are expressed as means  $\pm$  SD.

Comparisons made with controls by a Wilcoxon test for two samples \*  $p < 0.01$

Numbers in parentheses refer to the number of subjects in each group.

FIGURE 111.5:  $V_{\max}$  values for 5-HT uptake into platelets from untreated control subjects and unipolar depressed patients receiving mianserin



Values are expressed as means  $\pm$  SD.

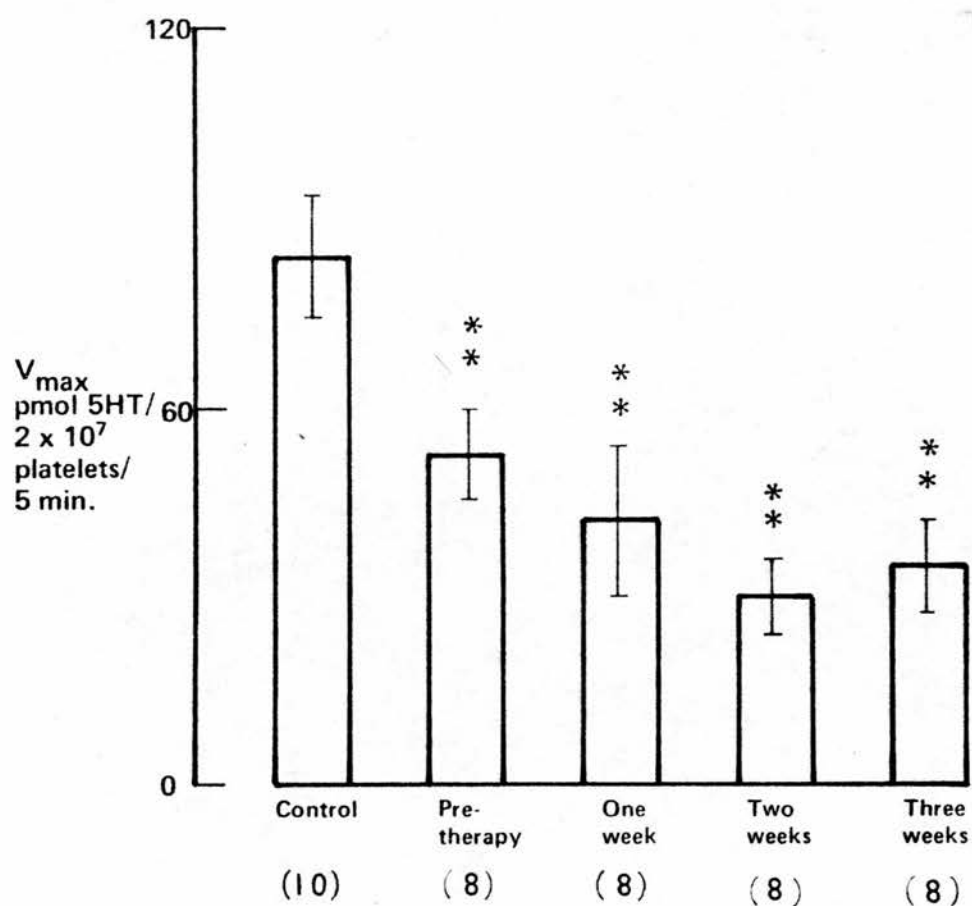
Comparisons made with controls by Wilcoxon test for two samples.

+  $P < 0.05$  \*  $P < 0.01$

Numbers in parentheses refer to the number of subjects in each group.



FIGURE 111.6:  $V_{\max}$  values for 5-HT uptake into platelets from untreated control subjects and unipolar depressed patients receiving amitriptyline



Values are expressed as means  $\pm$  SD.

Comparisons made with controls by Wilcoxon test for two samples.

\*  $P < 0.01$

Numbers in parentheses refer to the number of subjects in each group.

(c) Figures III.7 and III.8 show the *in vitro* effect of mianserin and amitriptyline, respectively, on  $K_m$  values for 5-HT uptake into platelets from control subjects. Mianserin causes a significant increase in  $K_m$  for the process at concentrations of 50 and 75 ng/ml. Amitriptyline causes an increase in  $K_m$  values at 50, 100 and 150 ng/ml. The effect produced by 100 ng/ml, however, is not significantly different from that produced by 150 ng/ml (Wilcoxon test for pair differences).

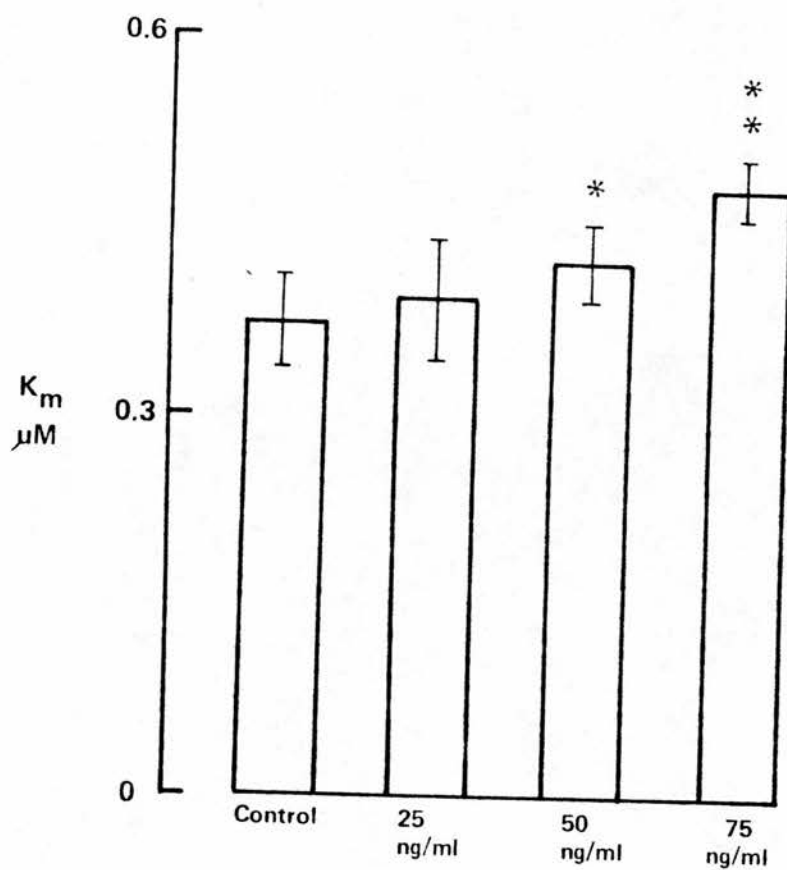
The *in vitro* action of mianserin and amitriptyline on  $V_{max}$  for 5-HT uptake into platelets from controls, is shown in Figures III.9 and III.10. Both mianserin and amitriptyline cause a reduction in the  $V_{max}$  values, at the concentrations shown.

(d) Some of the subjects already studied (Table III.26) were used to investigate the effect of either mianserin or amitriptyline on platelet and erythrocyte membrane ATPase specific activity (Table III.27). All of the patients investigated here, had recovered after 3 weeks drug administration. The control group was significantly younger than either of the two patient groups.

Tables III.28 and III.29 show values obtained for platelet membrane ATPase specific activities in unipolar depressed patients being treated with either mianserin or amitriptyline. Pre-therapy values in both patient groups studied were not significantly different from those in control subjects, and neither mianserin therapy and recovery nor amitriptyline therapy and recovery affected ATPase activity.

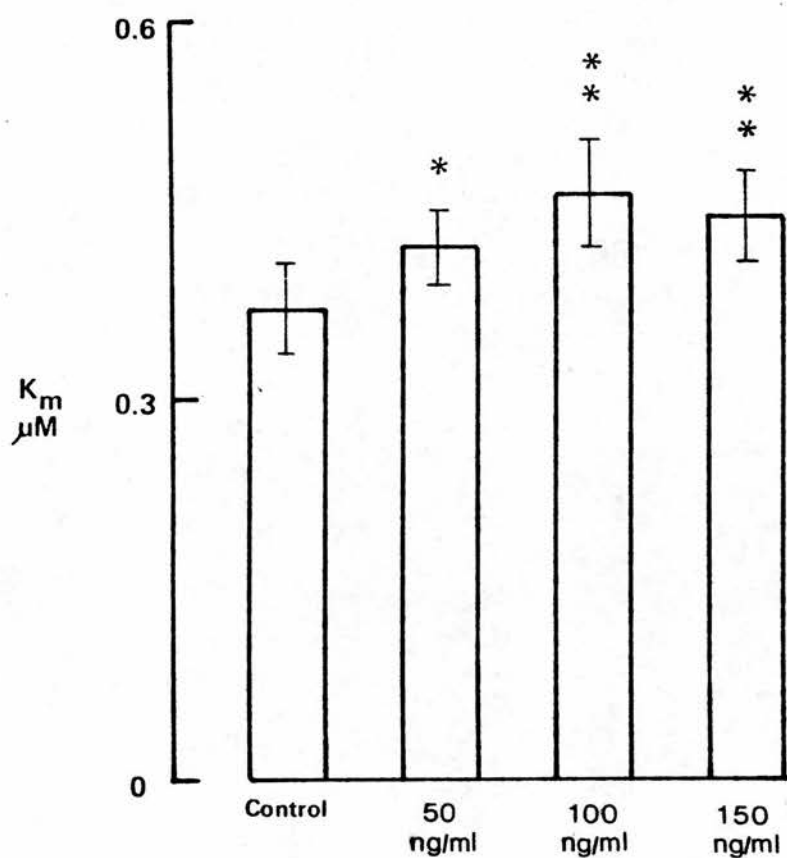
ATPase specific activities of erythrocyte membranes from patients treated with mianserin and amitriptyline are shown in

FIGURE III.7: In vitro action of mianserin on  $K_m$  values for 5-HT uptake into platelets from 10 control subjects



Values are expressed as means  $\pm$  SD.  
 Comparisons made with control value by a Wilcoxon  
 test for pair differences.  
 \*  $P < 0.02$     \*\*  $P < 0.01$

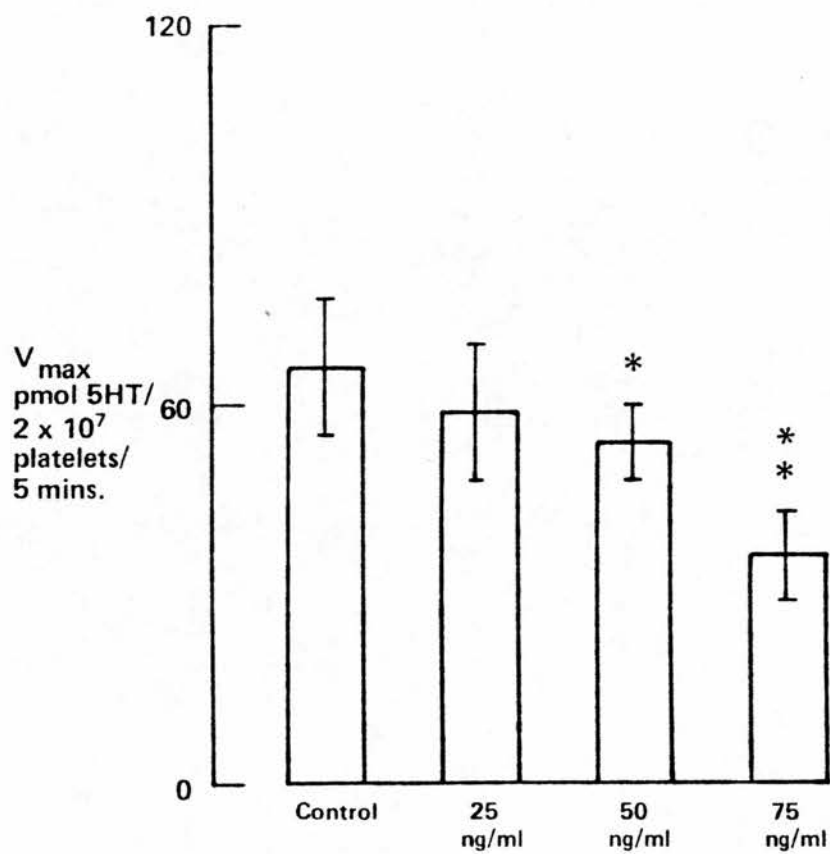
FIGURE III.8: In vitro action of amitriptyline on  $K_m$  values for 5-HT uptake into platelets from 10 control subjects



Values are expressed as means  $\pm$  SD.  
 Comparisons made with control value by a Wilcoxon test for pair differences.

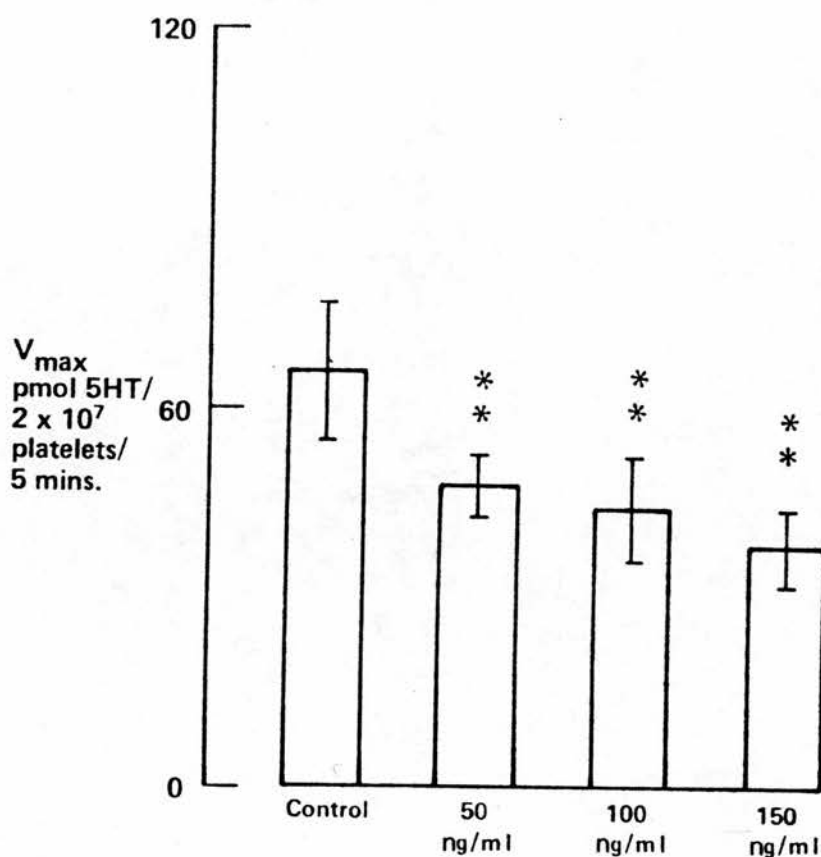
\*  $P < 0.02$     \*\*  $P < 0.01$

FIGURE III.9: In vitro action of mianserin on  $V_{max}$  values for 5-HT uptake into platelets from 10 control subjects



Values are expressed as means  $\pm$  SD.  
Comparisons made with control value by a Wilcoxon test for pair differences.  
\*  $P < 0.02$     \*\*  $P < 0.01$

FIGURE III.10: In vitro action of amitriptyline on  $V_{max}$  values for 5-HT uptake into platelets from 10 control subjects



Values are expressed as means  $\pm$  SD.  
 Comparisons made with control value by a Wilcoxon test for pair differences.  
 \*  $P < 0.01$

TABLE III.27: Group, age and sex of participating controls and patients

Drug therapy group	Estimations performed	Age	Sex
Controls (8)	Platelet membrane ATPase activity	$27 \pm 19$	4F 4M
	Erythrocyte membrane ATPase activity		
	<i>In vitro</i> action of mianserin and amitriptyline on platelet membrane and erythrocyte membrane ATPase activity		
Mianserin (8)	Platelet membrane ATPase activity	$46 \pm 15^*$	6F 2M
	Erythrocyte membrane ATPase activity		
Amitriptyline (5)	Platelet membrane ATPase activity	$40 \pm 12^*$	2F 3M
	Erythrocyte membrane ATPase activity		

Values are expressed as means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects in each group.

Comparisons made with control values by Wilcoxon test for two samples.

\*  $P < 0.02$

TABLE III.28: ATPase specific activities of platelet membranes from untreated control subjects and unipolar depressed patients receiving mianserin

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Control (8)	130 ± 21	752 ± 40
Pre-therapy (8)	142 ± 49	769 ± 70
1 week on mianserin (8)	136 ± 52	729 ± 68
2 weeks on mianserin (8)	127 ± 36	731 ± 59
3 weeks on mianserin (8)	151 ± 60	754 ± 65

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
 Values given are means ± SD.  
 Numbers in parentheses refer to the number of subjects at each stage.



TABLE III.29: ATPase specific activities of platelet membranes from untreated control subjects and unipolar depressed patients receiving amitriptyline

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Control (8)	130 ± 21	752 ± 40
Pre-therapy (5)	128 ± 40	689 ± 63
1 week on amitriptyline (5)	139 ± 37	699 ± 72
2 weeks on amitriptyline (5)	126 ± 52	696 ± 62
3 weeks on amitriptyline (5)	140 ± 48	709 ± 70

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.  
Numbers in parentheses refer to the number of subjects at each stage.

Tables III.30 and III.31, respectively. Pre-therapy  $\text{Na}^+ + \text{K}^+$ -ATPase activities in erythrocyte membranes from the patients were less than those in control subjects. Over the course of treatment with either mianserin or amitriptyline, and subsequent recovery, ATPase activities approached control values. No differences were detected in the other ATPase activities measured.

(e) The *in vitro* action of mianserin and amitriptyline on platelet and erythrocyte membrane ATPase specific activities was examined in preparations from control subjects.

Neither mianserin nor amitriptyline affected platelet membrane ATPase activities in the concentrations shown (Tables III.32 and III.33).

The action of mianserin and amitriptyline *in vitro* on erythrocyte membrane ATPase activities is shown in Tables III.34 and III.35. It can be seen that neither mianserin nor amitriptyline, in the concentrations used, change ATPase activity.

TABLE III.30: ATPase specific activities of erythrocyte membranes from untreated control subjects and unipolar depressed patients receiving mianserin

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Control (8)	360 ± 53	321 ± 57	900 ± 84
Pre-therapy (8)	216 ± 24 <sup>‡</sup>	299 ± 46	872 ± 69
1 week on mianserin (8)	223 ± 39 <sup>‡</sup>	330 ± 58	897 ± 70
2 weeks on mianserin (8)	272 ± 49 <sup>*</sup>	287 ± 53	921 ± 76
3 weeks on mianserin (8)	341 ± 60	294 ± 60	914 ± 93

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made with controls by Wilcoxon test for two samples.

<sup>‡</sup> P < 0.01      \* P < 0.02

Numbers in parentheses refer to the number of subjects in each group.

TABLE III.31: ATPase specific activities of erythrocyte membranes from untreated control subjects and unipolar depressed patients receiving amitriptyline

Stage of treatment	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Control (8)	360 ± 53	321 ± 57	900 ± 84
Pre-therapy (5)	254 ± 40 <sup>‡</sup>	339 ± 49	896 ± 60
1 week on amitriptyline (5)	269 ± 50 <sup>*</sup>	316 ± 36	935 ± 71
2 weeks on amitriptyline (5)	292 ± 43 <sup>+</sup>	349 ± 60	883 ± 54
3 weeks on amitriptyline (5)	323 ± 70	299 ± 58	899 ± 67

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made with controls by Wilcoxon test for two samples.

<sup>‡</sup> P < 0.01    \* P < 0.02    + P < 0.05

Numbers in parentheses refer to the number of subjects in each group.

TABLE III.32: In vitro action of mianserin on ATPase specific activities of platelet membranes from 8 control subjects

Concentration of mianserin present (ng/ml)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Control	137 ± 36	759 ± 38
25	146 ± 39	770 ± 39
50	129 ± 40	769 ± 37
75	132 ± 37	770 ± 40

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

TABLE III.33: *In vitro* action of amitriptyline on ATPase specific activities of platelet membranes from 8 control subjects

Concentration of amitriptyline present (ng/ml)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Control	137 ± 36	759 ± 38
50	132 ± 40	765 ± 29
100	143 ± 42	760 ± 32
150	151 ± 40	758 ± 30

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

TABLE III.34: In vitro action of mianserin on ATPase specific activities of erythrocyte membranes from 8 control subjects

Concentration of mianserin present (ng/ml)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Control	360 ± 53	321 ± 57	900 ± 84
25	342 ± 49	300 ± 48	892 ± 79
50	339 ± 50	299 ± 47	897 ± 82
75	351 ± 48	318 ± 49	905 ± 79

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

TABLE III.35: In vitro action of amitriptyline on ATPase specific activities of erythrocyte membranes from 8 control subjects

Concentration of amitriptyline present (ng/ml)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> + Mg <sup>2+</sup> - ATPase activity
Control	360 ± 53	321 ± 57	900 ± 84
50	352 ± 49	329 ± 53	911 ± 80
100	349 ± 53	320 ± 54	899 ± 81
150	369 ± 51	329 ± 51	901 ± 83

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.



#### 4. ELECTROCONVULSIVE THERAPY (ECT) STUDY

ATPase specific activities of erythrocyte membranes were examined in a group of unipolar depressed patients receiving a course of ECT (details given in Table III.36), a group of patients undergoing cystoscopy (four women and seven men; age  $49 \pm 19$  years) and a group of control subjects (13 women and three men; age  $45 \pm 11$  years).

(a) Figure III.11 shows ATPase values from control subjects, patients directly before cystoscopy and the total unipolar depressed patient group before ECT.  $\text{Na}^+ + \text{K}^+$  - ATPase values in pre-ECT patients were significantly reduced compared with control values. The mean Hamilton Rating Scale score for these patients, before ECT, was  $26 \pm 8$ . This score is consistent with clinical observations of depression shown by all the patients.

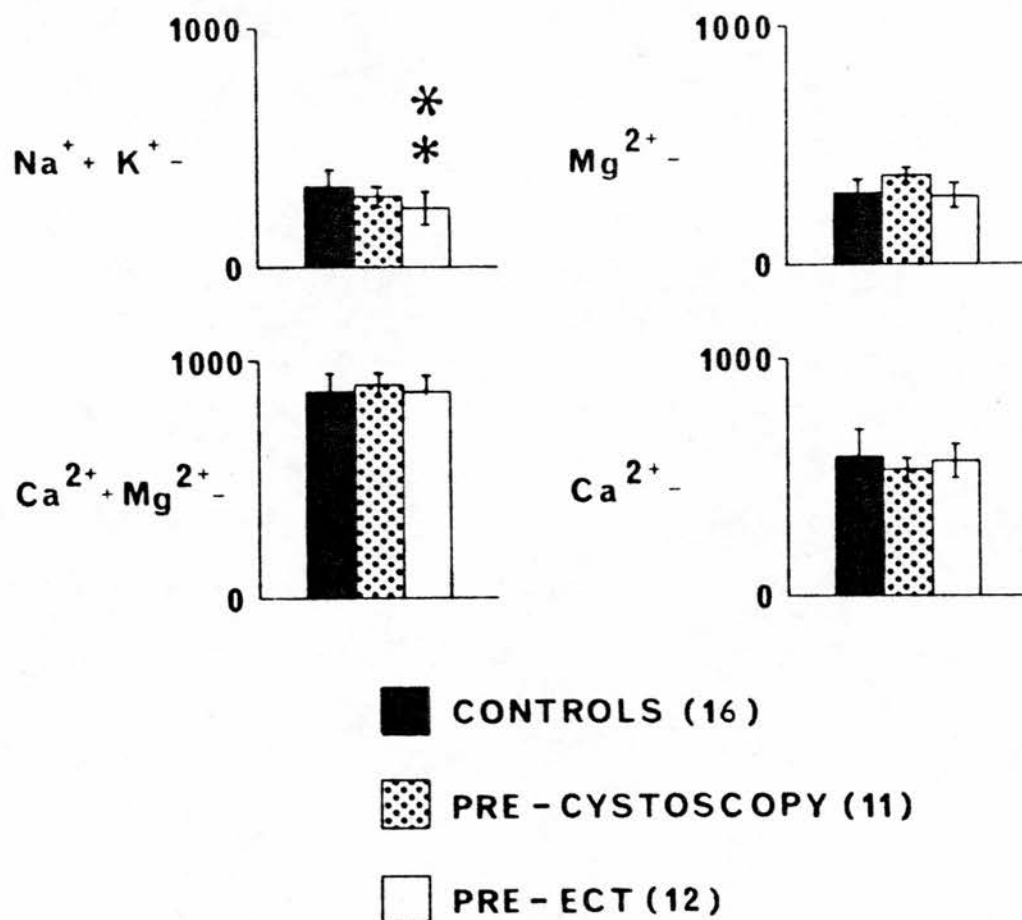
(b) Figure III.12 shows ATPase activities in the cystoscopy group before and after examination (anaesthetic given), and the total group of depressed patients before and after their first ECT. The only change observed was an apparent stimulation of  $\text{Na}^+ + \text{K}^+$  - ATPase activity in the cystoscopy group following anaesthesia.

(c) Results obtained from patient group I (subjects A, C, D, H, L, M, N, P and Q, seven women and two men; age  $50 \pm 15$  years) for ATPase activities before and after their first, second and last ECT are shown in Figure III.13. A slight reduction in  $\text{Ca}^{2+}$  - ATPase activity occurred after their first ECT. Prior to ECT, this group's mean HRS score was  $23 \pm 6$ , and immediately after a course of ECT this was lowered to  $11 \pm 4$ , a change consistent with the clinical

TABLE III.36: Details of patients receiving ECT

Patient	Sex	Age	No. of ECT	Hamilton Rating Scale:			Medication received during course of ECT
				Pre-ECT	Post-ECT	Follow-up	
A	M	40	6	27	17	24	amitriptyline
C	F	30	6	17	13	-	-
D	F	56	9	19	18	died	-
F	F	50	4	29	11	7	amitriptyline
H	F	39	8	28	10	10	-
J	F	36	5	37	17	9	amitriptyline
K	M	55	5	40	12	18	amitriptyline
L	F	65	8	31	7	10	amitriptyline
M	F	67	6	15	5	9	imipramine
N	M	57	6	23	8	4	amitriptyline
P	F	28	6	29	10	12	amitriptyline
Q	F	65	5	21	9	5	amitriptyline
MEAN		49	6	26	11	11	
SD		14	1.5	8	4	6	

FIGURE 11.11: ATPase specific activities of erythrocyte membranes from controls, patients before cystoscopy and depressed patients before ECT



Activities are expressed as nmol  $\text{P}_i$ /hr/mg protein.

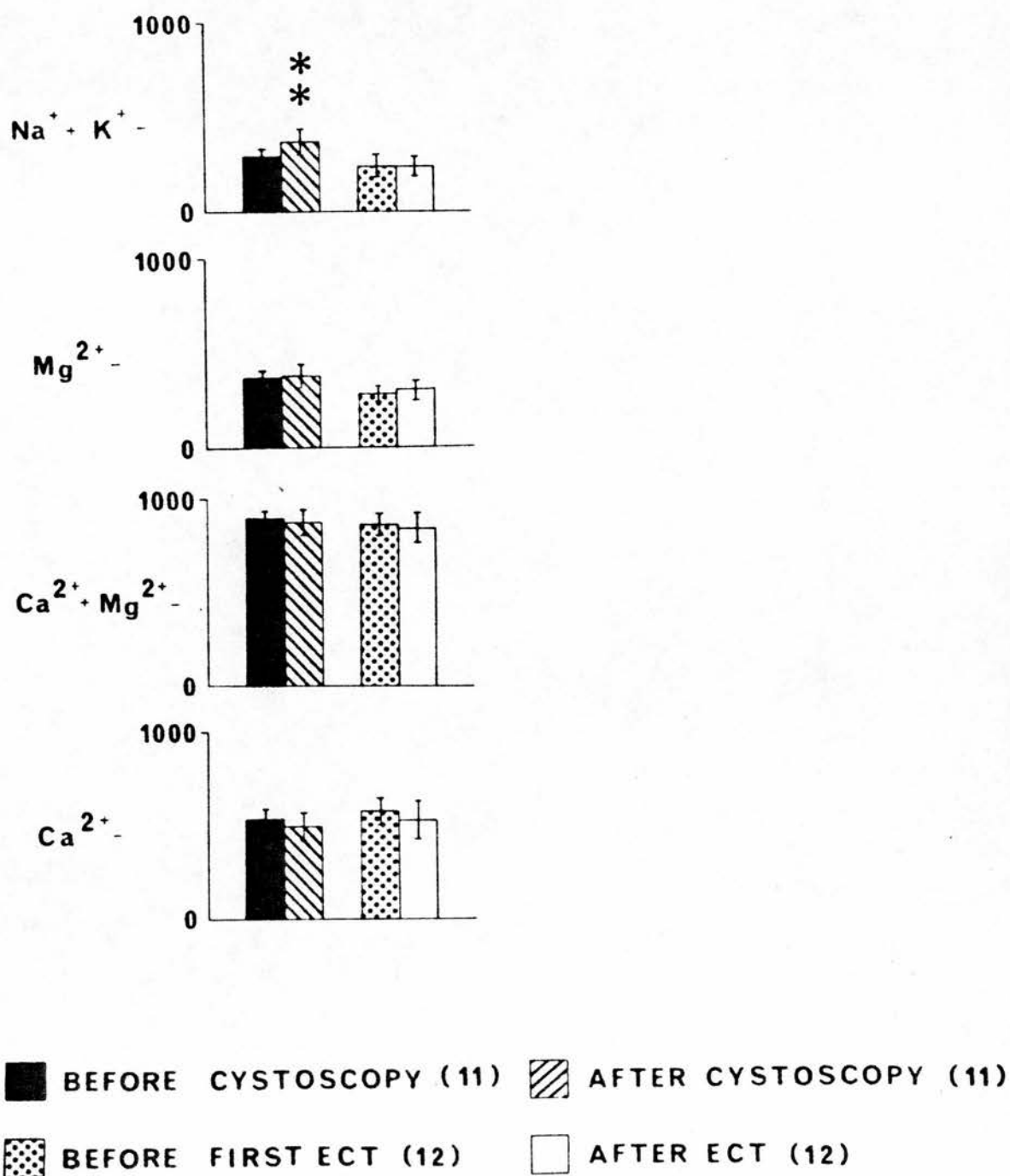
Values given are means  $\pm$  SD.

Comparisons made with control values by Wilcoxon test for two samples.

\*  $P < 0.01$

Numbers in parentheses refer to the number of subjects in each group.

FIGURE III.12: ATPase specific activities of erythrocyte membranes from patients before and after cystoscopy and from the total depressed patient group before and after their first ECT



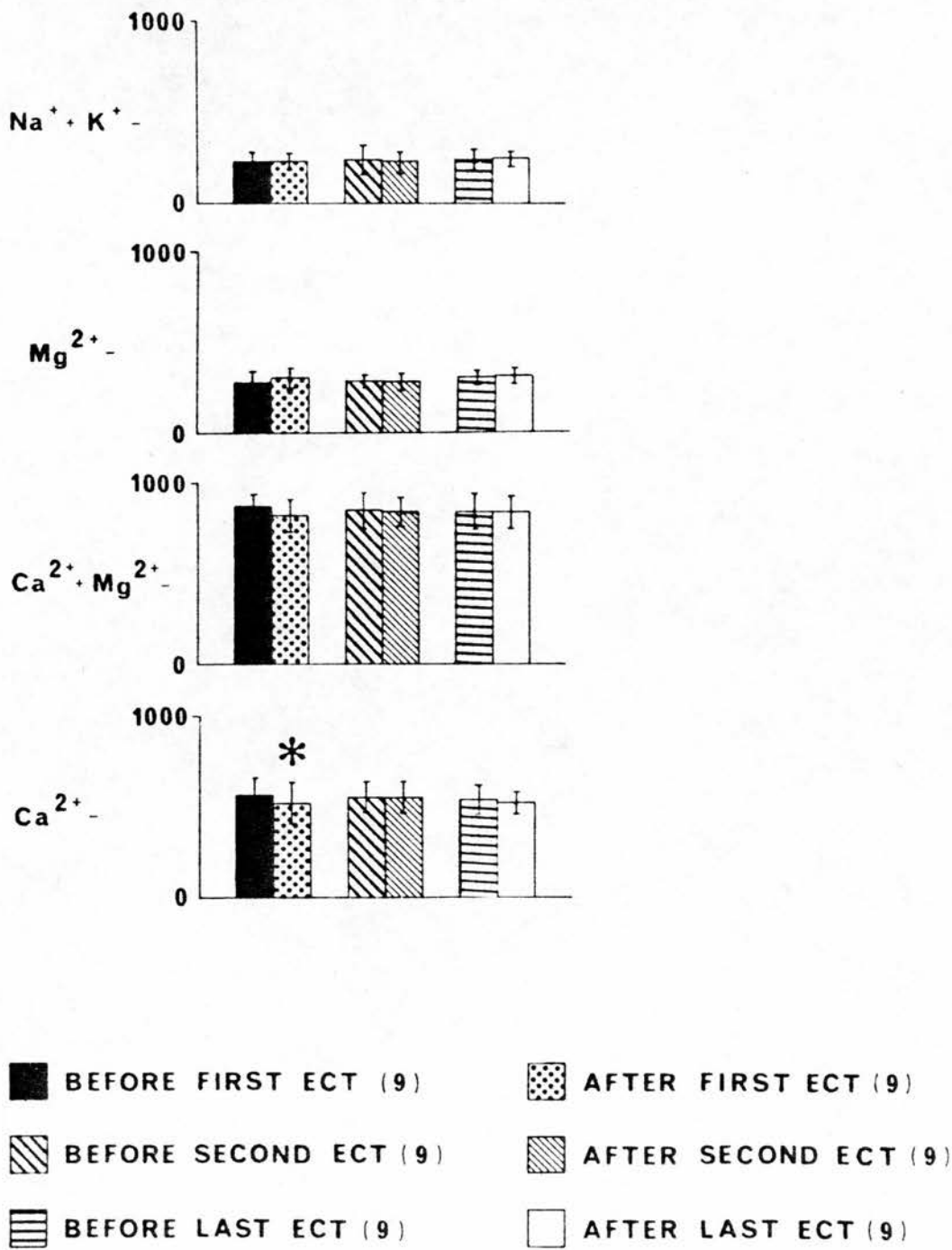
Activities are expressed as nmol Pi/hr/mg protein.  
 Values given are means  $\pm$  SD.

Comparisons made by Wilcoxon test for pair differences.

\* P < 0.01

Numbers in parentheses refer to the number of subjects in each group.

FIGURE III.13: ATPase specific activities of erythrocyte membranes from depressed patient group I before and after their first, second and last ECT.

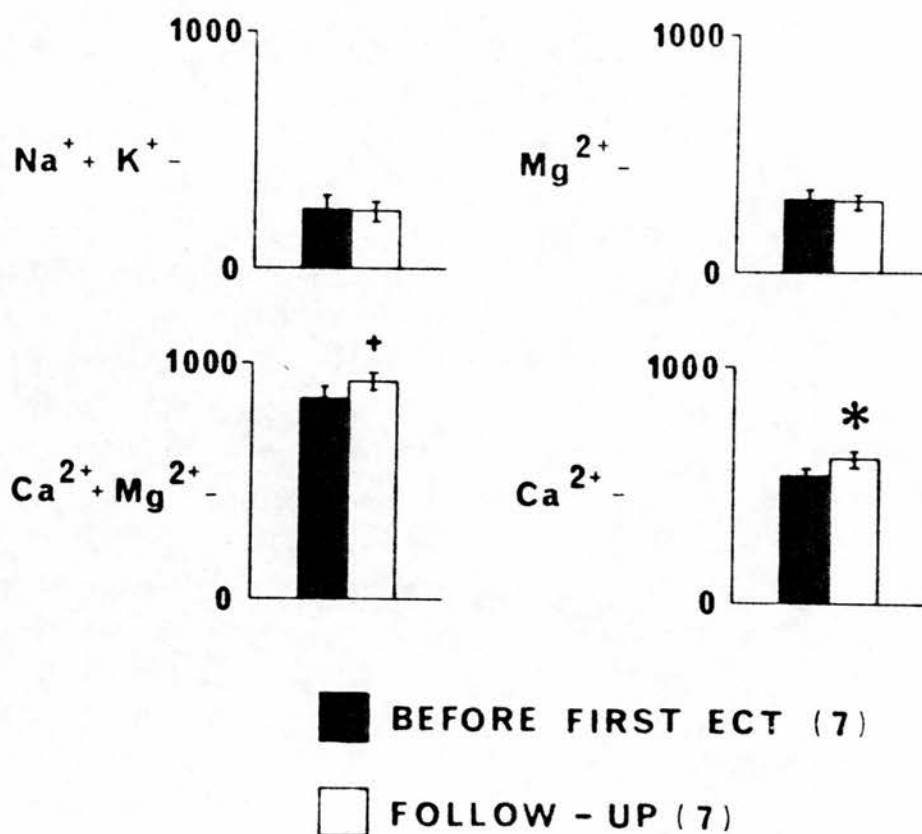


Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
 Values given are means ± SD.  
 Comparisons made by Wilcoxon test for pair differences.  
 \* P < 0.02  
 Numbers in parentheses refer to the number of subjects in each group.

improvement observed during the treatment. This group received  $7 \pm 1.3$  ECT.

(d) Figure III.14 shows values from patient group II (subjects F, H, J, K, N, P and Q, five women and two men; age  $47 \pm 13$  years) for ATPase activities before the first ECT and at follow-up. A small increase in  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity was observed. This observed increase was due entirely to an increase in  $\text{Ca}^{2+}$  - ATPase activity. In group II HRS scores were: before ECT  $29 \pm 7$ , immediately after a course of ECT  $11 \pm 3$ , and at follow-up 12 weeks later  $9 \pm 5$ . These scores parallel the clinical observations of improvement shown by these patients during the course of ECT. This group received  $6 \pm 1.3$  ECT.

FIGURE III.14: ATPase specific activities of erythrocyte membranes from depressed patient group II before their first ECT and at follow-up



Activities are expressed as nmol  $\text{P}_i$ /hr/mg protein.  
 Values given are means  $\pm$  SD.  
 Comparisons made by Wilcoxon test for pair differences.  
 \*  $P < 0.02$     +  $P < 0.05$   
 Numbers in parentheses refer to the number of subjects in each group.

## 5. ARRHENIUS PLOTS OF $\text{Na}^+ + \text{K}^+$ - ATPase ACTIVITY AND OUABAIN BINDING IN ERYTHROCYTES

ATPase specific activities in erythrocyte membranes from untreated unipolar depressed patients and controls, were measured at different temperatures, which enabled Arrhenius plots to be constructed, thus allowing calculation of activation energies for the enzyme. Ouabain binding was also measured in erythrocyte membranes from these subjects. Table III.37 gives details of the groups of subjects investigated (patients in the unipolar depressed group II are also represented in the unipolar depressed group I). Group I was significantly older than the control group.

(a) Table III.38 gives values for  $\text{Na}^+ + \text{K}^+$  - ATPase activities, measured at  $37^\circ\text{C}$ , from the three groups studied. Patient group I has a significantly reduced  $\text{Na}^+ + \text{K}^+$  - ATPase activity, while patient group II has a value which is slightly reduced compared to the control value.

(b) Apparent activation energies of  $\text{Na}^+ + \text{K}^+$  - ATPase (obtained from Arrhenius plots) of erythrocyte membranes from control subjects and unipolar depressed patient group I are shown in Table III.39. The Arrhenius plots for the ATPase activities gave two straight lines which intersected at about  $25^\circ\text{C}$ . Activation energies were calculated for each subject using both lines which gave values for the reaction above  $25^\circ\text{C}$  and values below  $25^\circ\text{C}$ . The values for the unipolar depressed patients do not differ significantly from those of the control subjects.



TABLE III.37: Groups investigated and estimations performed

Subjects	Estimations performed	Age	Sex
Controls (8)	Erythrocyte membrane $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ activities at different temperatures for Arrhenius plots. Ouabain binding in erythrocyte membranes.	$39 \pm 17$	5F 3M
Unipolar depressed patients I (10)	Erythrocyte membrane $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ activities at different temperatures for Arrhenius plots.	$51 \pm 15^*$	6F 4M
Unipolar depressed patients II (4)	Erythrocyte membrane $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ activity. Ouabain binding in erythrocyte membranes.	$48 \pm 13$	2F 2M

Values are expressed as means  $\pm$  SD.

Numbers in parentheses refer to the number of subjects in each group.

Comparisons made with control values by Wilcoxon test for two samples.

\*  $P < 0.02$

TABLE III.38: Na<sup>+</sup> + K<sup>+</sup> - ATPase specific activities of erythrocyte membranes from control subjects and untreated unipolar depressed patients

Group	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity
Controls (8)	356 ± 51
Unipolar depressed patients I (10)	200 ± 40‡
Unipolar depressed patients II (4)	296 ± 50+

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values are given as means ± SD.

Comparisons made with control value by a Wilcoxon test for two samples.

‡ P < 0.01    + P < 0.05

Numbers in parentheses refer to the number of subjects in each group.

TABLE III.39: Apparent activation energies of  $\text{Na}^+ + \text{K}^+$  - ATPase (obtained from Arrhenius plots) of erythrocyte membranes from control subjects and untreated unipolar depressed patients

Group	Apparent activation energy (E):	
	above 25°C	below 25°C
Controls (8)	16.5 ± 3.3	32.3 ± 5.7
Unipolar depressed patients I (10)	15.7 ± 1.9	29.5 ± 4.8

Activation energies are expressed as Kcal/mole. Values are given as means ± SD. Numbers in parentheses refer to the number of subjects in each group.

(c) Table III.40 shows specific ouabain binding in erythrocyte membranes from controls and the unipolar depressed patient group II. A Scatchard analysis of the binding gave a straight line indicating a single population of non-interacting sites. Ouabain binding is significantly reduced in the patient group compared to controls. Non-specific ouabain binding amounted to approximately 20% of the total binding.

Dissociation constants were also calculated using Scatchard plots. No difference in values was found to exist between controls and untreated patients (Table III.41).

TABLE III.40: Ouabain binding in erythrocyte membranes  
from control subjects and untreated  
unipolar depressed patients

Group	Ouabain binding
Controls (8)	521 $\pm$ 98
Unipolar depressed patients II (4)	323 $\pm$ 160*

Ouabain binding is expressed as  $\bar{x}$  moles ouabain bound/  
mg protein.

Values are given as means  $\pm$  SD.

Comparison made by a Wilcoxon test for two samples.

\*  $P < 0.02$

Numbers in parentheses refer to the number of subjects  
in each group.

TABLE III.41: Dissociation constant ( $K_D$ ) of ouabain -  
receptor complex in erythrocyte membranes  
from control subjects and untreated  
unipolar depressed patients

Group	Dissociation constant ( $K_D$ )
Controls (8)	$2.3 \pm 0.6$
Unipolar depressed patients (4)	$2.6 \pm 0.8$

$K_D$  is expressed in units of concentration (nM).  
Values are given as means  $\pm$  SD.  
Numbers in parentheses refer to the number of  
subjects in each group.

## 6. OTHER STUDIES

(a) ATPase activity was measured in platelet membranes, from 10 control subjects (5 female, 5 male; age  $31 \pm 8$  years), using incubation media that contained varying concentrations of magnesium, sodium, potassium and calcium.

Table III.42 shows  $Mg^{2+}$  - ATPase activities when the magnesium concentration of the media is varied, all other constituent concentrations remaining constant as detailed in Section II.  $Mg^{2+}$  - ATPase activity increases to a maximum when the magnesium concentration is between 6 and 10 mM. Increasing the concentration above that level causes a decrease in activity.

Table III.43 shows  $Na^{+} + K^{+}$  - ATPase activities when the concentrations of sodium and potassium in the incubation mixture were changed. Maximal activities are obtained when the sodium concentration is 100 mM and the potassium concentration is 5 mM.

$Ca^{2+}$  - ATPase activity was estimated in the presence of varying concentrations of calcium (Table III.44), but activity could not be detected in any of the preparations. Increasing the concentration of calcium caused a marked decrease in activity of  $Ca^{2+} + Mg^{2+}$  - ATPase activity (values not given), which in the platelet membrane preparation is equal to the  $Mg^{2+}$  - ATPase activity as no  $Ca^{2+}$  - ATPase activity is detected. This presumably was due to either a direct action of calcium on  $Mg^{2+}$  - ATPase activity or a change due to changing osmolarities of the incubation media.

TABLE III.42: Mg<sup>2+</sup>-ATPase specific activities of platelet membranes, in the presence of varying concentrations of Mg<sup>2+</sup> (all other incubation constituents kept constant), from 10 control subjects (5F, 5M)

Mg <sup>2+</sup> concentration (mM)	Mg <sup>2+</sup> - ATPase activity
1	200 ± 20
2	314 ± 39
3	400 ± 69
4	516 ± 54
5	570 ± 52
6	742 ± 60
10	750 ± 37
20	650 ± 59
30	526 ± 51

Activities are expressed as  
nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.



TABLE III.43: Na<sup>+</sup> + K<sup>+</sup> - ATPase specific activities of platelet membranes, in the presence of varying concentrations of Na<sup>+</sup> and K<sup>+</sup> (all other incubation constituents kept constant), from 10 control subjects (5F, 5M)

Na <sup>+</sup> concentration (mM)	K <sup>+</sup> concentration (mM)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity
50	-	68 ± 17
100	-	101 ± 30
150	-	69 ± 29
-	2.5	40 ± 10
-	5	37 ± 9
-	7.5	46 ± 17
50	2.5	98 ± 19
50	5	121 ± 34
50	7.5	149 ± 49
100	2.5	139 ± 21
100	5	179 ± 20
100	7.5	163 ± 14
150	2.5	153 ± 17
150	5	130 ± 21
150	7.5	101 ± 40

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

TABLE III.44: Ca<sup>2+</sup> - ATPase specific activities of platelet membranes, in the presence of varying concentrations of Ca<sup>2+</sup> (all other incubation constituents kept constant), from 10 control subjects (5F, 5M)

Ca <sup>2+</sup> - concentration (mM)	Ca <sup>2+</sup> - ATPase activity
0.05	nil
0.1	nil
0.15	nil
0.5	nil
1	nil
5	nil
10	nil
50	nil
100	nil

Activities are calculated as  
nmol P<sub>i</sub>/hr/mg protein.

(b) The effect of different anticoagulants; sodium citrate and heparin, on platelet and erythrocyte membrane ATPase activities was investigated in 10 control subjects (5 female, 5 male; age  $25 \pm 5$  years).

Table III.45 shows the ATPase activities found in platelets. Preparing the membranes from heparinized blood caused a marked reduction in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  and  $\text{Mg}^{2+} - \text{ATPase}$  activity compared with citrated blood.  $\text{Ca}^{2+} - \text{ATPase}$  activity was not detected in platelet membranes from either sodium citrated or heparinized blood. Protein content in the platelet membranes prepared from heparinized blood was greatly reduced compared with that in citrated blood (Table III.46).

ATPase activity and protein content of erythrocyte membranes from sodium citrated blood and heparinized blood were not significantly different (Tables III.47 and III.48).

(c) Altering the method of preparation of platelet membranes causes a change in the ATPase activity measured (Table III.49). Values shown are from 6 control subjects (4 female and 2 male; age  $36 \pm 7$  years). Maximal activity can be measured when 100  $\mu\text{l}$  Triton X100 and 2 - ml water are added and the preparation is frozen and thawed twice (the method routinely used for platelet membrane preparation). The  $\text{Mg}^{2+} - \text{ATPase}$  activity measured does not seem to be affected by the addition of Triton X100 or freezing and thawing, however  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activities are increased by these procedures. Adding 200  $\mu\text{l}$  Triton X100 results in the same level of activities as that produced by 100  $\mu\text{l}$  additions.  $\text{Ca}^{2+} - \text{ATPase}$  activity could not be detected in any of the preparations.

TABLE III.45: ATPase specific activities of platelet membranes, prepared from blood anticoagulated with either sodium citrate or heparin, from 10 control subjects (5F, 5M)

Preparation	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> - ATPase activity
Sodium citrated blood	163 ± 29	769 ± 50	nil
Heparinized blood	54 ± 22 <sup>*</sup>	642 ± 69 <sup>‡</sup>	nil

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made by Wilcoxon test for pair differences.

<sup>\*</sup> P < 0.01

TABLE III.46: Protein content of platelet membranes, prepared from blood anticoagulated with either sodium citrate or heparin, from 10 control subjects (5F, 5M)

Preparation	Protein content $\mu\text{g protein/ml}$
Sodium citrated blood	$355 \pm 30$
Heparinized blood	$110 \pm 46^{\ast}$

Values given are means  $\pm$  SD.

Comparison made by Wilcoxon test for pair differences.

$^{\ast}$   $P < 0.01$

Concentrations are expressed as  $\mu\text{g}$  platelet membrane protein per ml whole blood.

TABLE III.47: ATPase specific activities of erythrocyte membranes, prepared from blood anticoagulated with either sodium citrate or heparin, from 10 control subjects (5F, 5M)

Preparation	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> - ATPase activity
Sodium citrated blood	354 ± 44	300 ± 58	597 ± 60
Heparinized blood	348 ± 42	309 ± 54	589 ± 58

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.

TABLE III.48: Protein content of erythrocyte membranes, prepared from blood anticoagulated with either sodium citrate or heparin, from 10 control subjects (5F, 5M)

Preparation	Protein content μg protein/ml
Sodium citrated blood	986 ± 89
Heparinized blood	972 ± 95

Values given are means ± SD.  
Concentrations are expressed as μg erythrocyte membrane protein per ml whole blood.

TABLE III.49: Effect of change in platelet membrane preparation on ATPase specific activities of platelet membranes from 6 control subjects (4F, 2M)

Method of preparation:			Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
Triton X100 added	Water added	Freezing- thawing		
-	2 - ml	twice	83 ± 23	739 ± 29
-	2 - ml	once	86 ± 19	740 ± 32
-	2 - ml	-	69 ± 21	738 ± 36
100 µl	2 - ml	twice	127 ± 20	750 ± 31
100 µl	2 - ml	once	101 ± 20	746 ± 29
100 µl	2 - ml	-	96 ± 19	732 ± 30
200 µl	2 - ml	twice	129 ± 18	739 ± 28
200 µl	2 - ml	once	113 ± 19	752 ± 31
200 µl	2 - ml	-	100 ± 22	741 ± 29

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Values given are means ± SD.



(d) Haemoglobin and protein content of platelet and erythrocyte membrane preparations from 6 control subjects (4 female, 2 male; age  $36 \pm 7$  years) is shown in Table III.50. Haemoglobin amounts to approximately 6% of the total protein content in the platelet preparation and about 5% of the total in the erythrocyte preparation.

(e) ATPase specific activities of platelet membranes and intact washed platelets from 10 control subjects (5 females, 5 males; age  $42 \pm 9$  years) are shown in Table III.51.  $\text{Ca}^{2+}$ -ATPase activity could not be detected.  $\text{Na}^{+} + \text{K}^{+}$ -ATPase and  $\text{Mg}^{2+}$ -ATPase activities are significantly less in the intact washed platelets than in the platelet membrane preparation ('intact values' are approximately 37% and 10% respectively of the 'membrane values').

(f) Electron micrographs shown are typical examples obtained from samples processed from 35 control subjects (15 female, 20 male; age  $39 \pm 10$  years).

Figures III.19 and III.20 show electron micrographs of isolated intact platelets. Figure III.21 shows an enlargement of an intact platelet present in Figure III.19. 5-HT containing granules, glycogen particles and pseudopodia can be identified.

Platelet membrane preparations (routinely prepared; 100  $\mu\text{l}$  Triton X100 added, frozen and thawed twice) were also examined. Figure III.22 shows that the preparation is well disrupted (compare with Figures III.19 and III.20). Not all fields viewed in the electron microscope, of these preparations, showed quite as much disruption. Figure III.23 shows a typical example. The 5-HT granules are easily identified in this micrograph, having a characteristic 'bulls-eye' appearance.

TABLE III.50: Haemoglobin and protein content of platelet and erythrocyte membrane preparations from 6 control subjects (4F, 2M)

Preparation	Haemoglobin content $\mu\text{g/ml}$	Protein content $\mu\text{g/ml}$
Platelet membranes	$21 \pm 7$	$368 \pm 42$
Erythrocyte membranes	$50 \pm 12$	$965 \pm 89$

Values given are means  $\pm$  SD.

Concentrations are expressed as  $\mu\text{g}$  haemoglobin or protein per ml whole blood.

TABLE III.51: ATPase specific activities of platelet membranes and intact washed platelets from 10 control subjects (5F, 5M)

Preparation	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> - ATPase activity
Platelet membranes	124 ± 42	732 ± 69	nil
Intact washed platelets	46 ± 15*	76 ± 26*	nil

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Values given are means ± SD.

Comparisons made by Wilcoxon test for pair differences.

\* P < 0.01

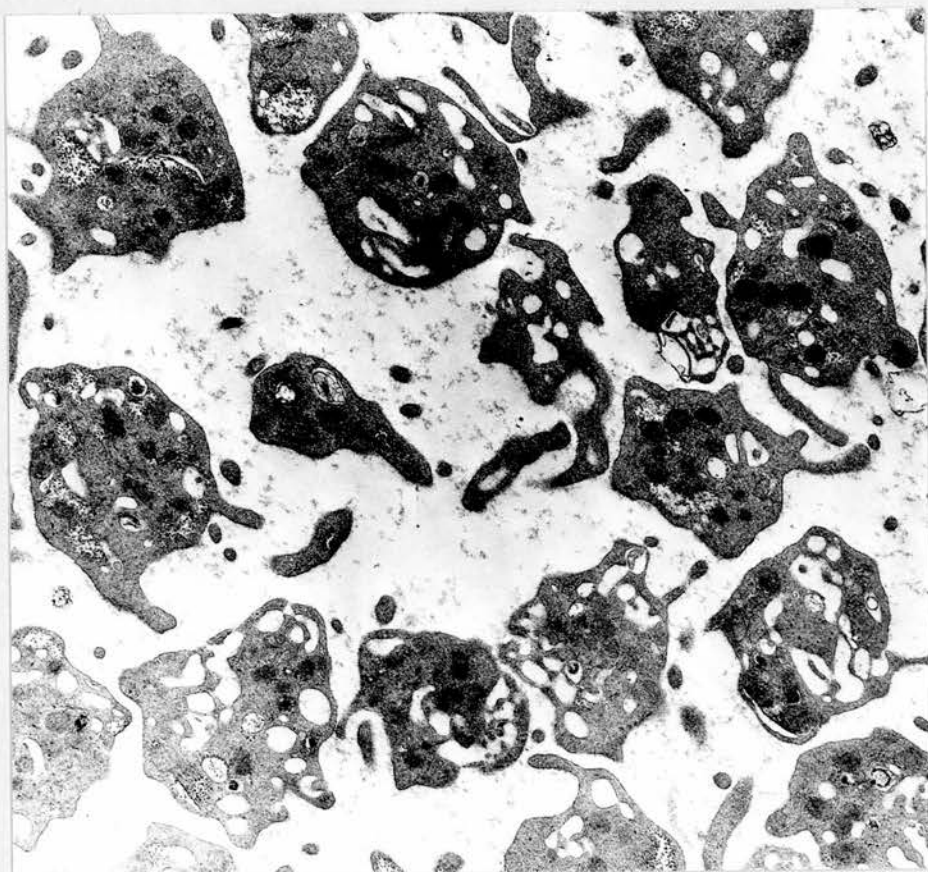


FIGURE III.19: Electron micrograph of isolated intact human platelets  
Magnification x 20,550

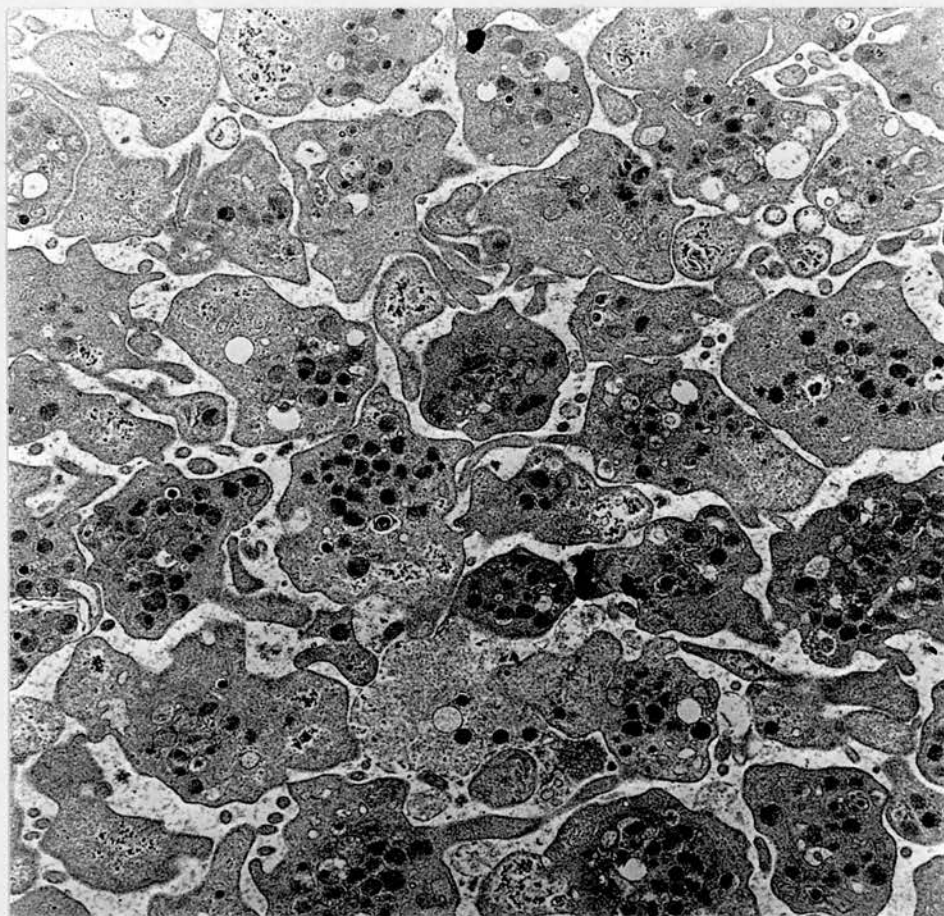


FIGURE III.20: Electron micrograph of isolated intact human platelets

Magnification x 11,600

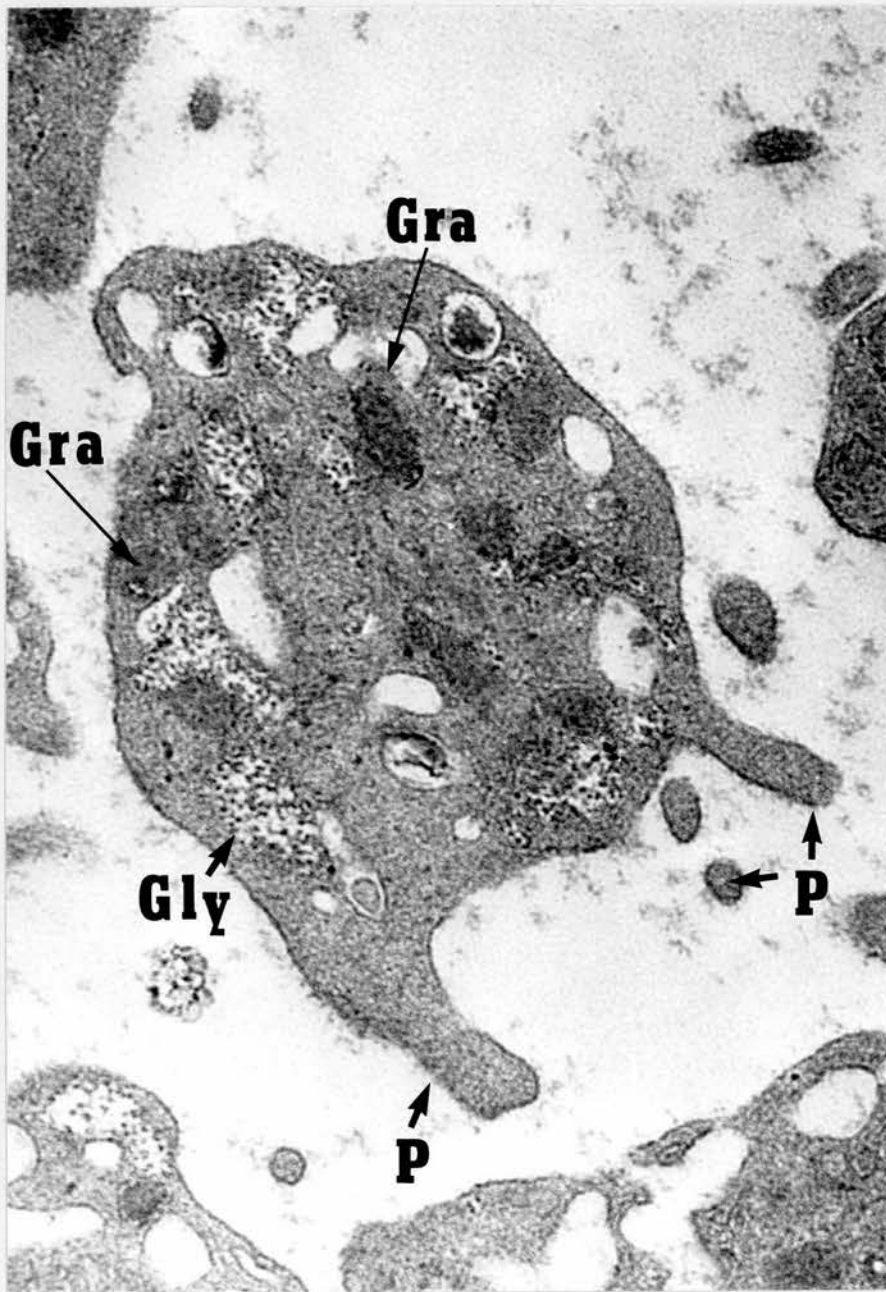


FIGURE III.21: Electron micrograph of an isolated intact human platelet. 5-HT granules (Gra), glycogen particles (Gly) and pseudopodia (P) can be identified  
Magnification x 104,850



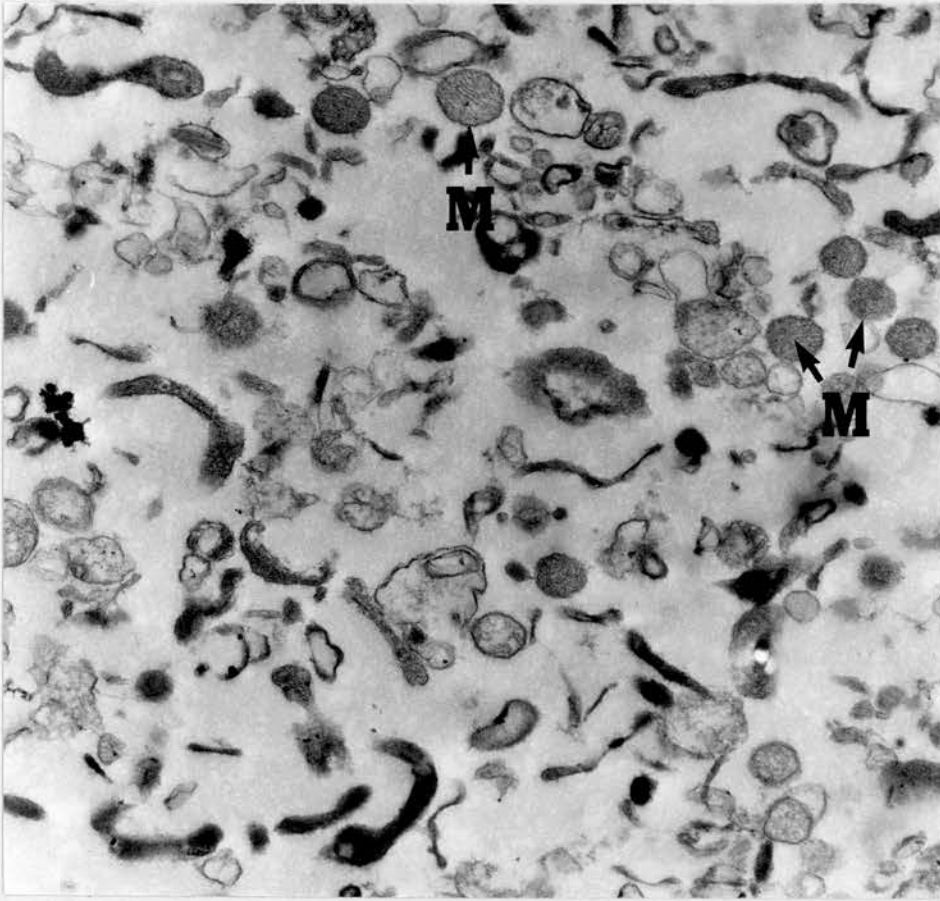


FIGURE III.22: Electron micrograph of human platelet membrane preparation. Mitochondria can be identified (M)  
Magnification x 69,000

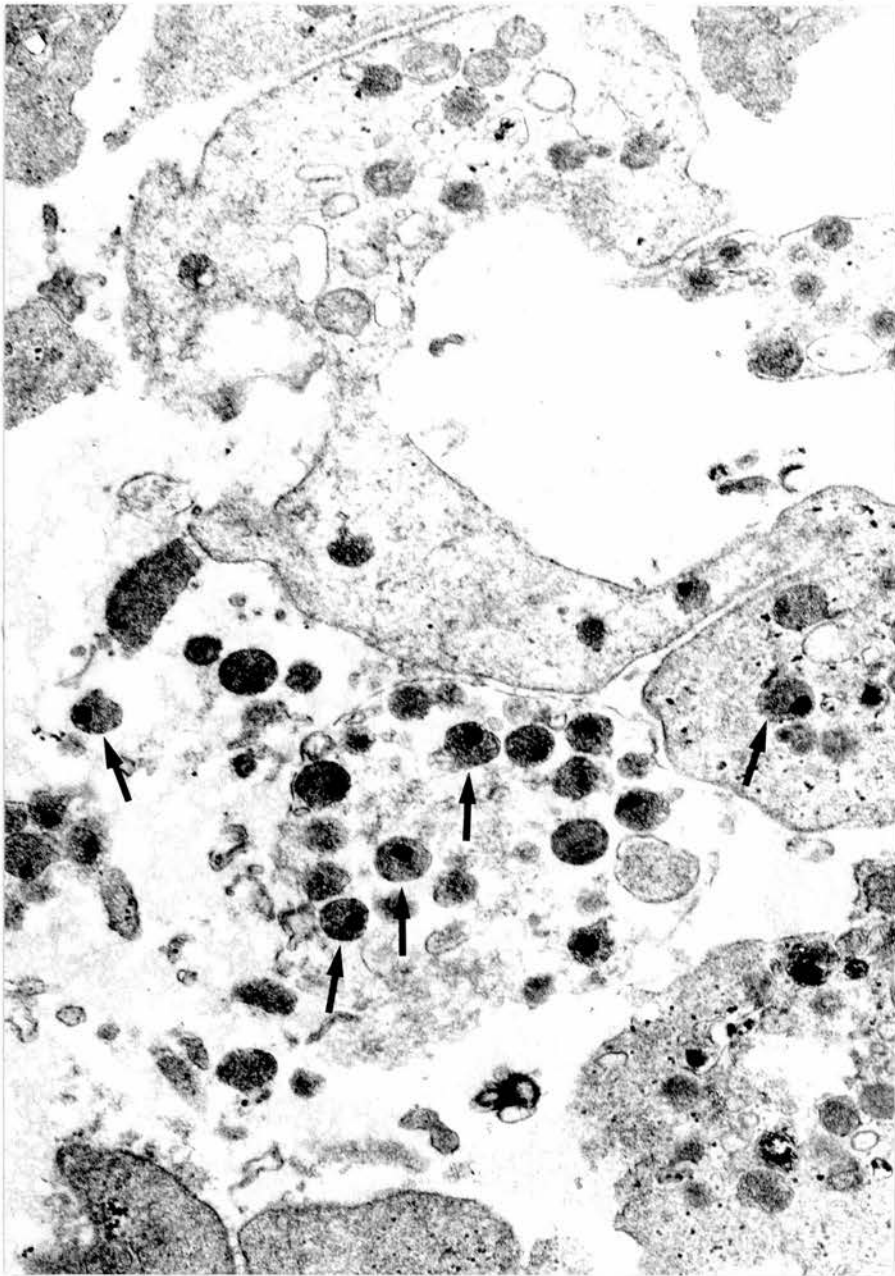


FIGURE III.23: Electron micrograph of human platelet membrane preparation with 5-HT granules arrowed  
Magnification x 66,500



(g) The variation which occurred in various platelet and erythrocyte parameters was examined in a female control subject over the course of a year (subject was 22 years old at start of study).

i) Tables III.52 and III.53 show seasonal and menstrual cycle variation, respectively, in ATPase activities in platelet membranes.

In Table III.53 day 1 refers to the day on which menstruation started. There appears to be more variation in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity during one menstruation cycle than in different seasons of the year.  $\text{Mg}^{2+} - \text{ATPase}$  activities do not seem to vary much.

Tables III.54 and III.55 show seasonal and menstrual cycle variation in ATPase activities in erythrocyte membranes. There again appears to be more variation during the menstrual cycle in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activities than from season to season.  $\text{Mg}^{2+} - \text{ATPase}$  and  $\text{Ca}^{2+} - \text{ATPase}$  activities vary during the menstrual cycle but not to quite the same extent as does the  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity.  $\text{Ca}^{2+} - \text{ATPase}$  activities do not seem to vary much from month to month.  $\text{Mg}^{2+} - \text{ATPase}$  activities, however, show a slightly greater seasonal variation.

ii) A big variation during the menstrual cycle and seasonal fluctuations can be seen in whole blood 5-HT content (Tables III.56 and III.57).

iii) Variation during the menstrual cycle and seasonal fluctuation can be seen in 5-HT uptake and platelet counts (Tables III.58 and III.59).

It should be noted that when seasonal variation was examined, blood samples were taken from the subject at approximately the same point in the menstrual cycle in any month.

TABLE III.52: Seasonal variation of ATPase specific activities of platelet membranes from a female control subject

Month (1978)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
February	160 (100%)	772 (100%)
May	189 (118%)	769 (100%)
August	196 (122%)	756 (98%)
November	173 (108%)	763 (99%)

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Numbers in parentheses refer to the activity expressed as a percentage of the value obtained in February.

TABLE III.53: Variation of ATPase specific activities of platelet membranes during the menstrual cycle of a control subject

Day of blood sampling (April - May 1978)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity
1	153 (100%)	769 (100%)
6	109 (71%)	758 (98%)
13	200 (131%)	749 (97%)
22	296 (193%)	750 (97%)
30	149 (97%)	772 (100%)

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.

Numbers in parentheses refer to the activity expressed as a percentage of the value obtained on day 1 of the cycle.

TABLE III.54: Seasonal variation of ATPase specific activities of erythrocyte membranes from a female control subject

Month (1978)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> - ATPase activity
February	372 (100%)	259 (100%)	601 (100%)
May	297 (80%)	272 (105%)	589 (98%)
August	290 (78%)	321 (124%)	562 (93%)
November	329 (88%)	299 (115%)	578 (96%)

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein. Numbers in parentheses refer to the activity expressed as a percentage of the value obtained in February.

TABLE III.55: Variation of ATPase specific activities of erythrocyte membranes during the menstrual cycle of a control subject

Day of blood sampling (April - May 1978)	Na <sup>+</sup> + K <sup>+</sup> - ATPase activity	Mg <sup>2+</sup> - ATPase activity	Ca <sup>2+</sup> - ATPase activity
1	264 (100%)	246 (100%)	650 (100%)
6	287 (109%)	259 (105%)	609 (94%)
13	379 (143%)	272 (110%)	569 (87%)
22	300 (114%)	269 (109%)	532 (82%)
30	152 (57%)	276 (112%)	691 (106%)

Activities are expressed as nmol P<sub>i</sub>/hr/mg protein.  
Numbers in parentheses refer to the activity expressed as a percentage of the value obtained on day 1 of the cycle.

TABLE III.56: Seasonal variation of whole blood  
5-HT content in a female control  
subject

Month (1978)	5 - HT content (ng/ml)
January	209 (100%)
April	127 (61%)
June	98 (47%)
September	111 (53%)
November	117 (56%)

Numbers in parentheses refer to the concentration expressed as a percentage of the value obtained in January.

TABLE III.57: Variation of whole blood 5 - HT content during the menstrual cycle of a control subject

Day of blood sampling (November - December 1978)	5 - HT content (ng/ml)
1	112 (100%)
8	129 (115%)
15	209 (187%)
23	225 (201%)
30	105 (94%)

Numbers in parentheses refer to the concentration expressed as a percentage of the value obtained on day 1 of the cycle.

TABLE III.58: Seasonal variation of 5-HT uptake ( $K_m$  and  $V_{max}$  values) into platelets and platelet count in a female control subject

Month (1978)	$K_m$	$V_{max}$	Platelet count
January	0.44 (100%)	99 (100%)	389 (100%)
April	0.51 (116%)	87 (87%)	362 (93%)
June	0.47 (107%)	90 (91%)	299 (77%)
September	0.48 (109%)	89 (90%)	345 (89%)
November	0.43 (98%)	97 (98%)	309 (79%)

$K_m$  values are expressed in units of  $\mu M$ ,  
 $V_{max}$  in units of  $\text{pmol } 5\text{-HT} / 2 \times 10^7 \text{ platelets} / 5 \text{ mins}$   
and platelet counts as  $10^3 \text{ platelet} / \text{mm}^3 \text{ blood}$ .  
Numbers in parentheses refer to the activity or count  
expressed as a percentage of the value obtained in  
January.



TABLE III.59: Variation of 5-HT uptake ( $K_m$  and  $V_{max}$  values) into platelets, and platelet count, during the menstrual cycle of a control subject

Day of blood sampling (Nov-Dec 1978)	$K_m$	$V_{max}$	Platelet count
1	0.40 (100%)	89 (100%)	269 (100%)
8	0.39 (97%)	75 (84%)	273 (101%)
15	0.43 (107%)	63 (71%)	300 (111%)
23	0.47 (117%)	52 (58%)	309 (115%)
30	0.41 (102%)	61 (68%)	240 (89%)

$K_m$  values are expressed in units of  $\mu M$ ,  
 $V_{max}$  in units of  $\text{pmol } 5\text{-HT} / 2 \times 10^7 \text{ platelets} / 5 \text{ mins}$   
and platelet counts as  $10^3 \text{ platelets} / \text{mm}^3 \text{ blood}$ .  
Numbers in parentheses refer to the activity or count  
expressed as a percentage of the value obtained on day 1  
of the cycle.

SECTION IV

DISCUSSION

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## 1. PLATELET AND ERYTHROCYTE MEMBRANE ATPase ACTIVITIES

The results of the platelet study (described in Section III, part 1 of this thesis) show that blood platelets from patients with affective disorders do not differ to any great extent from those of corresponding control subjects in activities of the different ionic ATPases. This result is interesting in view of the proposal of Naylor *et al* (1973) that depression involves a general decrease in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity. These workers found that an increase in erythrocyte membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity was associated with a recovery from depression. That erythrocyte membranes from depressed patients do indeed have a reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity, compared to control subjects, was shown by Hesketh *et al* (1977).

It was considered necessary to confirm the observations made in the platelet study by simultaneously examining platelet and erythrocyte membrane ATPase activities from the same patients (Section III, part 2 of this thesis). In this study the number of groups investigated was restricted. A unipolar depressed group of patients was included because Hesketh *et al* (1977) had found the most marked reduction in erythrocyte membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in this group. It was hoped that by selecting such a group, the probability would be increased of detecting any  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  abnormality. A bipolar manic group was selected for study because an ATPase activity change was not expected in these patients and it was hoped that they might act as hospitalized controls. However, bipolar manics are not a group that have been investigated much, probably because of the difficulty in obtaining blood samples from

them and the need to administer treatment quickly. The inclusion of this group, provided in any case, valuable additional information. The finding in this study that there is a reduced activity in erythrocyte membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  confirmed the work reported by Hesketh *et al* (1977). It was shown, once again, that patients' platelet membrane ATPase activities seemed no different from those of control subjects.

A reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in erythrocyte membranes from unipolar depressed patients was also found in three other studies reported in this thesis. 111 patients prior to mianserin or amitriptyline therapy (Section III, part 3 (d)), patients about to receive ECT (Section III, part 4) and a small group whose blood samples were used to investigate lipid content and ouabain binding in erythrocytes (Section III, part 5 (a)), all showed  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity less than normal. In the study reported in Section III, part 3 (d), platelet ATPase activities were also estimated. They were found to be no different from those in control subjects.

The question of why such a change occurs in the erythrocyte membrane and not in the platelet is to some extent unresolved. However, various explanations can be offered, but the most important deduction from these studies is that changes in ATPase activity in the erythrocyte membrane associated with depressive illness may not be general to all cell membranes in the body. This implies that detection of changes in a peripheral cell membrane and extrapolation of such changes to central neurones is still an open question.

There are, however, certain differences between the erythrocyte and the blood platelet membrane preparations used for ATPase assays.

Platelet membranes assayed in this study were heterogeneous in nature, having membrane components from the plasma membrane, mitochondria, granule membranes and dense tubular, microtubular and cannalicular systems. Separation and purification of subcellular components was not carried out, due to restrictions in the volume of blood sample which could be obtained. In fact, carrying out enzyme assays on separated platelet plasma membranes would require fairly large volumes of blood (Barber and Jamieson, 1970; Siegel, Burri, Weibel, Bettex-Galland and Lüscher, 1971); which would have been totally impracticable with this patient material. On the other hand, it is relatively easy to obtain sufficient isolated washed plasma membranes from erythrocytes from a sample of ten to twenty millilitres of blood.

It would appear from results presented in Section III, part 6, that optimal conditions were used for the estimation of  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity. The use of 6 mM  $\text{Mg}^{2+}$ , 100 mM  $\text{Na}^+$  and 5 mM  $\text{K}^+$  in the incubation medium would have been expected to produce maximal activity in any samples assayed.

Using sodium citrate as anticoagulant, compared with heparin, which was used when erythrocyte membranes were prepared, did not decrease observable  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity. In fact, it is interesting to observe that the use of heparin anticoagulant, when platelet membranes were to be prepared, produced quite a large reduction in platelet membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity. As the protein content was found to be reduced in such samples, it is possible that the difference observed is due to heparin being a less efficient anticoagulant. Platelets might therefore aggregate

more readily, and these aggregates might be spun down into the red blood cell fraction. Such an explanation necessitates that the platelets, left unaggregated in the platelet rich plasma, have a lower ATPase activity than those removed. Day, Scrutton and Holmsen (1973) have provided evidence that there are populations of platelets having differing contents of calcium and magnesium. It is possible there are also platelet populations showing differing ATPase activities. It may also be that heparin has a direct action on platelet ATPase activity, which persists even when the heparin is no longer in contact with the membranes. Heparin does not, however, seem to affect erythrocyte membrane ATPase activity. An anomaly exists in the observation that the protein content of the erythrocyte membrane fraction prepared from heparinized blood is not greater than that prepared from blood anticoagulated with sodium citrate. This might be expected due to contamination by platelet aggregates. However, it is quite possible that these aggregates are lighter than the erythrocytes, and are removed in the washing stages during the preparation of erythrocyte membranes.

The use of Triton X100, and the process of freezing and thawing, although not affecting platelet membrane  $Mg^{2+}$ -ATPase activity greatly, seemed to activate  $Na^{+}+K^{+}$ -ATPase activity. However, it should be noted that Triton X100 was not used to disrupt the erythrocyte membrane preparation. In view of the studies of Bramley, Coleman and Finean (1971) and Hanahan, Ekholm and Hildenbrandt (1973), who showed that the properties of the erythrocyte membrane preparation depended markedly on the conditions used during preparation, the procedure described in this thesis for the preparation of erythrocyte

membranes would be expected to produce membranes exhibiting their total ATPase capacity. Similar studies, however, have not been carried out using platelet membranes.

Platelet and erythrocyte membranes showed approximately the same degree of haemoglobin contamination. It seems unlikely, then, that haemoglobin could cause spurious differences between platelet and erythrocyte preparations.

$\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity could be detected in intact washed platelets. It is not known if this activity represents a true ecto-ATPase (i.e. an enzyme which splits ATP on the outer face of the cell membrane) or if it is due to the  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity of the sodium pump (i.e. the enzyme which splits ATP on the inner face of the cell membrane) in the platelet. It would be interesting to know if the intact washed platelets used had an increased permeability to ATP, which would enable sodium pump  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity to be observed. No estimate can be made, on the basis of the results given here, of the contribution of any true ecto-ATPase to  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity measured in disrupted platelets.

Electron micrographs of platelet membranes showed that the isolation procedures used produced samples which were well disrupted, which would hopefully thus allow full expression of any  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity present. However, it is interesting to consider the micrographs of intact platelets. The morphological appearance of these platelets (Figure III.19) is very similar to those used by Siegal *et al* (1971), prior to homogenization. Many pseudopodia are seen in both preparations. However, platelets prepared by Kinlough-Rathbone, Mustard, Packham, Perry, Reimers and Cazenave (1977) appear to have retained their normal disc shape. Such observed



differences may be due to differences in preparation of samples for electron microscopic analysis. It is worth considering, though, the possibility that platelet membranes used in studies described in this thesis, might have been prepared from platelets which had undergone the first stages of aggregation. It is quite possible that such transformations would influence the observed activity of an enzyme which is known to span the plasma membrane. It would have been useful to prepare platelet membranes from reversibly and irreversibly aggregated platelets to observe if a change in  $\text{Na}^+ + \text{K}^+$  - ATPase activity resulted.

Another factor may lie in the different relative distribution of the ATPases in the erythrocyte and platelet membrane. In the blood platelet the  $\text{Mg}^{2+}$  - / $\text{Na}^+ + \text{K}^+$  - ATPase ratio was found to be approximately 6 : 1, while in the erythrocyte the corresponding ratio was 1 : 1. It would appear that the relatively lower activity of  $\text{Na}^+ + \text{K}^+$  - ATPase does not necessarily prevent detection of activity changes occurring in illness, since McCoy, Segal, Bayer and Strynadka (1974) observed a reduction in  $\text{Na}^+ + \text{K}^+$  - ATPase activity in blood platelets from patients with Down's syndrome. Their preparation was also heterogeneous since ultrasonic disintegration was used to disrupt the platelet pellets and no subsequent purification was carried out. However, it should be noted that McCoy *et al* (1974) measured  $\text{Na}^+ + \text{K}^+$  - ATPase activity in the presence of sodium, potassium and magnesium. In terms of the conditions used in this thesis, such activity would be designated as 'total'. It is interesting to note, though, that McCoy *et al* (1974) detected a reduced intraplatelet potassium and an increased intraplatelet sodium concentration in

platelets from patients with Down's syndrome. These findings would suggest that  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity was in fact reduced in these platelets. Values obtained in this thesis for platelet ATPase activities are in approximate agreement with those obtained by other workers (Table IV.1). It should be noted that Moake *et al* (1970)

TABLE IV.1 Lysed platelet ATPase activities

	ATPase activities nmol $\text{P}_i$ /hr/mg protein:	
	$\text{Na}^+ + \text{K}^+ -$	$\text{Mg}^{2+} -$
Barber and Jamieson, 1970	121	-
Moake, Ahmed, Bachur and Gutfreund, 1970	380	1280

measured  $\text{Mg}^{2+} - \text{ATPase}$  activity in the presence of  $\text{MgCl}_2$  and  $\text{NaCl}$ , whereas results in this thesis refer to activities in the presence of  $\text{MgCl}_2$  alone. Values given by McCoy *et al* (1974) could not be compared as these workers quoted their activities in units of nmoles  $\text{AT}^{32}\text{P/hr}/10^8$  platelets.

$\text{Ca}^{2+} - \text{ATPase}$  activity could not be detected with the platelet preparation used in this study. This enzyme activity has been measured in human platelets by several workers (Chambers, Salzman and Neri, 1967; Mason and Saba, 1969; Barber and Jamieson, 1970; French, Holmsen and Stormorken, 1970; Coffrey and Middleton, 1975). Intact washed platelets (Chambers *et al*, 1967; Mason and Saba, 1969; Coffrey and Middleton, 1975), pure membrane fractions from platelets which had been hypotonically lysed after loading with glycerol (Barber and Jamieson, 1970), lysates from platelet suspensions

which had been frozen and thawed, and subcellular fractions prepared from these lysates (French *et al*, 1970) and soluble and particulate fractions from platelet preparations which had been subjected to sonication (Coffrey and Middleton, 1975) were used by these workers in the estimation of ATPase activity.

These workers all used different anticoagulants. Chambers *et al* (1967) and Barber and Jamieson (1970) used acid citrate dextrose, which was also used by French *et al* (1970), with the addition of EDTA. The anticoagulant used by Mason and Saba (1969) contained trisodium citrate dihydrate, citric acid and glucose. Heparin was used by Coffrey and Middleton (1975).

Concentrations of ions present in the ATPase incubation media also varied between the groups. Chambers *et al* (1967) and Barber and Jamieson (1970) both used 2.5 mM  $Mg^{2+}$ , 1.25 mM  $Ca^{2+}$  and 1  $\mu$ M ATP. Mason and Saba (1969) state in their paper that concentrations of  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ ,  $K^+$  and ATP were varied to determine optimal conditions, but unfortunately they do not state what those optimal conditions were. French *et al* (1970) used either 10 mM  $Ca^{2+}$  or  $Mg^{2+}$  and 400  $\mu$ M ATP. Coffrey and Middleton (1975) employed concentrations of 5 mM  $Ca^{2+}$  or  $Mg^{2+}$  and 2 mM ATP. It is interesting to note that French *et al* (1970) found that the ATPase activity in platelet lysates in the presence of  $Ca^{2+}$  and  $Mg^{2+}$  together, was less than with either ion separately. This suggested to them "that one enzyme and not two major ATPases were involved". They found that increasing concentrations of  $Mg^{2+}$ , acting alone, produced an increase in enzymic activity up to 6 mM  $Mg^{2+}$ , and above this concentration no change was observed. In contrast, increasing concentrations of  $Ca^{2+}$

were found to give no significant change below 5 mM. Above 5 mM, the ATPase activity increased with increasing  $\text{Ca}^{2+}$  concentration, and did not level off below 15 mM. However, Chambers *et al* (1967) found that in intact washed platelets, in the presence of  $\text{Mg}^{2+}$ , addition of  $\text{Ca}^{2+}$ , stimulated enzyme activity. Both groups found that  $\text{Mg}^{2+}$  alone stimulated activity more than  $\text{Ca}^{2+}$  alone. It is interesting to note that Chambers *et al* (1967) investigated the action of 1.25 mM  $\text{Ca}^{2+}$  at only three  $\text{Mg}^{2+}$  concentrations, namely: 1.25, 2.5 and 5 mM. From their results it would appear that (5 mM  $\text{Mg}^{2+}$  + 1.25 mM  $\text{Ca}^{2+}$ ) produced less activity than (2.5 mM  $\text{Mg}^{2+}$  + 1.25 mM  $\text{Ca}^{2+}$ ). It may be that if this group had used higher magnesium concentrations they would have got results similar to those of French *et al* (1970). It is also possible that the two groups are monitoring activities of two distinct ATPases.

As Chambers *et al* (1967) give their ATPase activities in cpm and in moles  $\text{P}_i/\text{hr}/\text{ml}$  and Mason and Saba (1969) give their values in units of  $\text{mg P}_i/\text{hr}/4 \times 10^8$  platelets, it is rather hard to compare their results with those presented in this thesis. There exists some confusion in the results quoted by Barber and Jamieson (1970). It is not clear from their paper if values quoted are for  $\text{Mg}^{2+}$ - or  $\text{Ca}^{2+}$ -ATPase activities, so once again a comparison is difficult. However, values quoted by Coffrey *et al* (1975), for non-asthmatic children, can be readily compared with activities quoted in this thesis (Table IV.2). It can be seen that the value Coffrey and Middleton (1975) obtain for  $\text{Mg}^{2+}$ -ATPase activity in sonicated platelets is approximately half the value reported in this thesis for platelet membranes, and a quarter of that reported by Moake

TABLE IV.2: ATPase activities measured in platelet preparations by Coffrey and Middleton (1975)

	Mean ATPase activity nmol P <sub>i</sub> /hr/mg protein
Intact platelet Mg <sup>2+</sup> - ATPase	160
Intact platelet Ca <sup>2+</sup> - ATPase	100
Sonicated platelet Mg <sup>2+</sup> - ATPase	328
Sonicated platelet Ca <sup>2+</sup> - ATPase	208

*et al* (1970). The subjects used by Coffrey *et al* (1975) were children. This may be the reason for the discrepancy. Values obtained by French *et al* (1970) for Mg<sup>2+</sup> - ATPase activity are in better agreement (Table IV.3). The value reported by French *et al*

TABLE IV.3: ATPase activities measured in platelet lysates by French *et al* (1970)

	ATPase activity nmol P <sub>i</sub> /hr/mg protein
No divalent cations present	224
10 mM Mg <sup>2+</sup>	876
10 mM Ca <sup>2+</sup>	399
10 mM Mg <sup>2+</sup> + 10 mM Ca <sup>2+</sup>	241
8 mM EDTA	28

(1970) for Ca<sup>2+</sup> - ATPase activity is slightly higher than that reported by Coffrey and Middleton (1975). This may be due to the slightly different Ca<sup>2+</sup> concentrations used in each case. It is interesting that the value obtained by French *et al* (1970) when no divalent cations were present, and presumably no Na<sup>+</sup> or K<sup>+</sup> either,

is very similar to the values reported in this thesis for  $\text{Na}^+ + \text{K}^+$  - ATPase activities. Activities of platelet  $\text{Na}^+ + \text{K}^+$  - ATPase reported in this thesis are definitely sensitive to  $\text{Na}^+$  and  $\text{K}^+$ , as shown in Section III, part 6 (a). It is possible to compare results obtained by Chambers *et al* (1967) with those obtained by Mason and Saba (1969) (Table IV.4), after recalculation of their data. Chambers

TABLE IV.4:  $\text{Ca}^{2+}$  - ATPase activities in intact washed platelets

Chambers <i>et al</i> (1967)	1270 nmol $\text{P}_i$ /hr/ $6 \times 10^5$ platelets 640 nmol $\text{P}_i$ /hr/ $5 \times 10^5$ platelets
Mason and Saba (1969)	7789 nmol $\text{P}_i$ /hr/ $4 \times 10^8$ platelets

*et al* (1967) report two different values in their paper which are 70 to 100 times greater than the activity quoted by Mason and Saba (1969). It can be seen that there exists some variation in values quoted by workers, which may be due to differences in preparation of the platelet samples and in the ATPase assay conditions employed.

It has been suggested that the  $\text{Ca}^{2+}$  - and  $\text{Mg}^{2+}$  - stimulated ATPase activity present in the platelet is due to the presence of thrombosthenin, a contractile protein (Bettex-Galland and Lüscher, 1961; Hanson, Repke, Katz and Aledort, 1973). Bettex-Galland and Lüscher (1961) have shown that thrombosthenin purified from human blood platelets is activated by magnesium and by calcium; the latter having the more pronounced effect. Hanson *et al* (1973), however, measured activity in either the presence of  $\text{MgCl}_2$  and  $\text{CaCl}_2$  together (138 nmol  $\text{P}_i$ /hr/mg protein) or  $\text{MgCl}_2$  alone (108 nmol  $\text{P}_i$ /hr/mg protein). It is somewhat surprising that a purified thrombosthenin

preparation does not have a higher specific activity when one considers values obtained in platelet lysates by other workers. There exists some controversy as to the function of these ATPases. Holmsen, Day and Stormorken (1969) consider there to be an intimate relationship between thrombosthenin and the explosive platelet release reaction. Other workers have suggested that ecto-ATPases may play a part in cell adhesion. Jones (1966) and Salzman, Chambers and Neri (1966) have suggested that aggregation or adhesion occurs when a membrane bound contractile protein relaxes due to a decrease in its ATPase activity. However, Robinson, Kress, Wagner and Brinkhous (1965) consider that aggregation is due to an increase in ATPase activity which produces a decrease in membrane bound ATP and a resultant decrease in surface charge of the platelet. Several workers, using sulphhydryl inhibitors, ouabain, DTNB and DNP (Mason and Saba, 1969; Saba, Rodman and Mason, 1969) have suggested that there may be several ATPases present, but they are uncertain as to which is the most important in aggregation. It has also been suggested that the  $\text{Ca}^{2+}$ -ATPase activity in the human platelet reflects the presence of a calcium membrane pump (Robblee, Shepro and Belamarich, 1973).

It is interesting to speculate why  $\text{Ca}^{2+}$ -ATPase activity could not be detected in the platelets of either patients with affective illness or control subjects. Changing the concentration used in the incubation medium (Table III.44), using a different anticoagulant (Table III.45), changing the platelet membrane preparation (Table III.49) and using intact washed platelets (Table III.51) failed to reveal any  $\text{Ca}^{2+}$ -ATPase activity. It should be pointed out that in



all assays performed, when  $\text{Ca}^{2+}$  was used in the incubation medium,  $\text{Mg}^{2+}$  was always present at a concentration of 6 mM. It is possible that if  $\text{Mg}^{2+}$  had been omitted,  $\text{Ca}^{2+}$  - ATPase activity may have been uncovered.

It can be appreciated that controversy arises because of the relatively complex nature of the platelet. However, erythrocytes can be used to produce membrane fractions which are free from contamination by subcellular organelles. It is well established that the  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity present in the erythrocyte membrane is associated with active transport of sodium and potassium (Dunham and Glynn, 1961; Skou, 1965; Whittam and Wheeler, 1970). In addition to this ATPase activity dependant on  $\text{Mg}^{2+}$ , stimulated by sodium and potassium and sensitive to ouabain, Dunham and Glynn (1961) also detected an activity dependant on magnesium alone, which was insensitive to ouabain. In addition they found calcium to cause a stimulation of the ouabain-insensitive activity. Such magnesium dependant ( $\text{Mg}^{2+}$  - ATPase) and calcium stimulated magnesium dependant activities ( $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase) were also reported by Wins and Schoffeniels (1966). As pointed out by Wins and Schoffeniels (1966), the physiological function of the  $\text{Mg}^{2+}$  - ATPase is unknown. It could possibly reflect adenylyl cyclase activity since adenylyl cyclase produces pyrophosphate which might be detected in the analysis of inorganic phosphate.

Using potassium iodide fractionation Nakao, Nagano, Adachi and Nakao (1963) were able to obtain two preparations from erythrocyte membranes. One possessed  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity, which was sensitive to ouabain, and the other contained an ouabain-insensitive



$\text{Mg}^{2+}$  - ATPase which was not stimulated by sodium or potassium. These workers suggested that the  $\text{Na}^{+} + \text{K}^{+}$  - ATPase and  $\text{Mg}^{2+}$  - ATPase were separate enzymes.

Drickamer (1975) studied ATPases by examining erythrocyte membrane protein phosphorylation in the presence of magnesium alone, calcium and magnesium, and finally sodium, potassium and magnesium. Using gel electrophoresis he was able to separate three different phosphorylated proteins corresponding to the different ionic conditions. He concluded that the three ATPases were distinct enzymes. The available evidence suggests that in the erythrocyte,  $\text{Mg}^{2+}$  - ATPase,  $\text{Na}^{+} + \text{K}^{+}$  - ATPase and  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase are distinct enzymes.

It has been suggested that the  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase and the calcium pump of the erythrocyte membrane are manifestations of the same system (Schatzmann, 1975). However, it has also been proposed that this enzyme is associated with actomyosin-like protein in erythrocyte membranes (Wins and Schoffeniels, 1966). The physiological function of this ATPase might be to maintain the biconcave disc shape of the red blood cell.

$\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity could be measured in erythrocyte membranes from patients and control subjects, although such activity was not observed in their platelet membranes. A reduction in  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity was observed in erythrocyte membranes from bipolar manics (Section III, part 2).  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity was found to be normal in all groups of unipolar depressed patients examined. Considering the importance of calcium in cell function, this observation is interesting. Calcium plays a crucial role in neurotransmitter release (Katz and Miledi, 1970; Rahamimoff,

Rahamimoff, Binah and Meiri, 1975). However, it seems unlikely that  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity in the erythrocyte could reflect events concerning calcium movements in neuronal tissue (i.e. in the brain). It is not known from the estimations performed, if the change observed in  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity, was due to a change in the activity of the calcium pump or of the proposed contractile protein of the erythrocyte.

The effects of various therapies on platelet and erythrocyte membrane ATPase activities were investigated. Hesketh *et al* (1977, 1978) had found that in unipolar depressed patients with reduced erythrocyte membrane  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity, long term treatment with lithium and subsequent recovery, caused the activities to return to normal. They also found that  $\text{Mg}^{2+}$  - ATPase activity in the erythrocyte membranes was increased above control values, after lithium therapy. It was shown by these workers that the change in activity of  $\text{Na}^{+} + \text{K}^{+}$  - ATPase was due to the recovery process, and that the  $\text{Mg}^{2+}$  - ATPase change was solely a drug effect, i.e. lithium-treated patients who had not recovered from their depression had reduced  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activities. Hesketh *et al* (1978) had found changes in  $\text{Mg}^{2+}$  - ATPase activities after three weeks of lithium administration, when recovery in these patients "was at most only partial". At this time no change was observed in  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity.

It had been hoped to extend Hesketh's observations, and possibly monitor an earlier change, by studying platelets at five days, three weeks and three months after lithium therapy had commenced. However, it was shown that lithium administration and recovery did not appear

to influence platelet ATPase activity. Although an action by lithium on platelet  $\text{Na}^+ + \text{K}^+$  - ATPase activity was perhaps not to be expected, as the activities were normal when the patients were ill, an effect on  $\text{Mg}^{2+}$  - ATPase activity might have been expected. Unfortunately, it was possible to investigate only a few patients receiving lithium. As a result, conclusions drawn from this part of the study can only be tentative. No change in platelet  $\text{Mg}^{2+}$  - ATPase activities was observed in control subjects taking lithium. This would affirm that lithium administration does not affect the  $\text{Mg}^{2+}$  - ATPase of the platelet. However, lithium in a concentration expected to be present in plasma therapeutically, produced an *in vitro* stimulation of  $\text{Mg}^{2+}$  - ATPase activity in platelet membranes from control subjects. This result is interesting in view of the fact that lithium *in vitro* has been shown to have no effect on  $\text{Mg}^{2+}$  - ATPase in other tissues (Gupta and Crollini, 1975). The stimulation shown by lithium would tend not to support the view that lithium exerts its effects by means of competition with magnesium for magnesium-dependant enzymes (Birch, 1973).

Neither mianserin nor amitriptyline was found to influence platelet ATPase activities when present *in vivo* or *in vitro*.

However, when erythrocyte membranes were prepared from unipolar depressed patients receiving either mianserin or amitriptyline, it was found that drug treatment and recovery resulted in  $\text{Na}^+ + \text{K}^+$  - ATPase activities approaching normal values. No effect was observed in the activities of the other ATPases present in the erythrocyte membrane. However, as all patients in this study recovered, it was not possible to dissociate drug effects from the recovery process, as Hesketh *et al* (1978) had done. Neither mianserin nor amitriptyline

was shown to affect erythrocyte membrane ATPase activity *in vitro*.

It is quite possible that in the groups studied the increase in activity of  $\text{Na}^+ + \text{K}^+$ -ATPase was due to the recovery process.

However, it should be remembered that the *in vitro* action of mianserin and amitriptyline was tested in preparations from control subjects, not patients with affective disorder.

It is interesting that in these studies a change in  $\text{Na}^+ + \text{K}^+$ -ATPase activity occurred over a period of about three weeks. Although Hesketh *et al* (1978) observed a stimulation of  $\text{Na}^+ + \text{K}^+$ -ATPase activity in erythrocyte membranes from depressed patients after long term treatment with lithium, such a change was not seen after 2 - 4 weeks of treatment.

Since the lifespan of the human erythrocyte is approximately 120 days in control subjects (Harris and Kellermeyer, 1970) and in patients with affective illness (Bille, Jensen, Kaalund-Jensen and Paulsen, 1975) the increase in  $\text{Na}^+ + \text{K}^+$ -ATPase activity observed after 3 weeks of treatment with either mianserin or amitriptyline suggests that the effect of recovery on the specific enzyme activity was not dependent on the synthesis of new red blood cells. This is also the conclusion Hesketh *et al* (1978) came to when they considered observed changes in  $\text{Mg}^{2+}$ -ATPase activities. The  $\text{Na}^+ + \text{K}^+$ -ATPase activity change observed here after 3 weeks may be due to activation of the enzyme by some unknown factor.

It is interesting to compare results presented here with those of Naylor, Buckley, Boardman, Smith and Moody (1978) who found that mianserin therapy did not affect erythrocyte membrane  $\text{Na}^+ + \text{K}^+$ -ATPase activity. This group carried out their biochemical estimations after

two weeks of treatment, at a time when their patients were "showing signs of clinical improvement". As Naylor *et al* (1978) did not investigate a control group at the same time, it is not known if these patients had a reduced erythrocyte membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity before treatment commenced.

When patients receiving ECT were examined, an increase in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity was not observed. It can be seen by the HRS scores of these patients, that most of the patients in this study improved during a course of ECT. This would tend to suggest that a stimulation of the reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in the erythrocyte membrane in unipolar depression is not necessarily related to the recovery process. The group of patients in this study had been selected for ECT because, for the main part, other therapies had been unsuccessful. It is possible that these patients, although classified as unipolar depressed, are distinct from those that are successfully treated with drugs alone.

It is useful to compare the results of this ECT study with those obtained by Choi *et al* (1977). This group studied 5 bipolar and 6 unipolar depressed patients. The activity of both  $\text{Na}^+ + \text{K}^+ -$  and  $\text{Ca}^{2+} - \text{ATPase}$  was found to be reduced in these patients. After a course of ECT, when all of their patients had at least moderately improved, the activity of these two enzymes was found to be indistinguishable from that in control subjects. A reduced  $\text{Ca}^{2+} - \text{ATPase}$  activity was not observed in the total patient group (A C D F H J K L M N P Q) or in group I or group II. However, results obtained for patient group II showed an increase in  $\text{Ca}^{2+} - \text{ATPase}$  activity twelve weeks after their last ECT. This increase was fairly

small in comparison to that obtained by Choi *et al*(1977) in patients after a successful course of ECT. It should be remembered that Choi *et al*(1977) investigated both unipolar and bipolar depressed patients, whereas results quoted in this thesis were obtained from only unipolar depressives. A reduction in  $\text{Ca}^{2+} + \text{Mg}^{2+}$  - ATPase activity was observed in erythrocyte membranes from bipolar manics (see Section 111, part 2 of this thesis).

It is difficult to determine, in this study, the effect of anaesthetic on the ATPase measurements. In the cystoscopy control group a stimulation in  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity was observed. No such acute stimulation was observed in the ECT group, and in fact patient group 1 showed a slight reduction in  $\text{Ca}^{2+}$  - ATPase activity after the first ECT. These observations probably highlight unfortunately the difference in the anaesthetic procedures used in the two groups. Psychiatric information was not available for the cystoscopy controls, a proportion of whom were suffering from serious medical conditions. They were all likely to be apprehensive before induction of anaesthesia, so unfortunately information obtained from this group will not reveal the effect of anaesthesia alone on erythrocyte membrane ATPase activity. However, it is interesting that over the period of approximately ten minutes a change in erythrocyte ATPase activity was observed. These short term changes may be due to rapid changes in the concentration of "enzyme modulators", as considered by Hesketh *et al*(1978).

As the only consistent finding in the ATPase studies was a reduced  $\text{Na}^{+} + \text{K}^{+}$  - ATPase activity in erythrocyte membranes in unipolar depressed patients, further studies were undertaken to try



and explain why such a decrease occurred. These studies involved estimating activation energies of the  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ , using Arrhenius plots. Discontinuities in Arrhenius plots of membrane bound enzyme activities have been correlated with lipid phase changes (Raison, Lyons, Mehlforn and Keith, 1971; Wisniesky, Parkes, Huang and Fox, 1974; Wynn-Williams, 1976) and it has been shown that  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity is sensitive to its lipid environment (Schwartz *et al*, 1975). It was hoped that if a change in lipid composition was responsible for the reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity, it would be detected in these plots. However, unipolar depressed patients seemed no different from controls. It is worthwhile to consider that fairly gross changes in lipid composition would be necessary before a change could be detected in a plot of this sort (see Taniguchi and Iida, 1972).

Ouabain binding was also estimated. A significant reduction in ouabain binding to erythrocyte membranes was observed with no concomitant change in the dissociation constant  $K_d$ . The value obtained for  $K_d$  in both control subjects and patients was very similar to that obtained by Erdmann and Hasse (1975) and indicated a binding site with high affinity for the drug. It is rather harder to compare the  $B_{\text{max}}$  (maximal number of membrane-bound ouabain binding sites) obtained in this thesis with that estimated by Erdmann and Hasse (1975). These workers reported their values as number of sites per single erythrocyte ( $228 \pm 28$  per single erythrocyte). Red blood cell counts were not performed in the studies reported in this thesis. However, taking the information given in Table 111.50 of this thesis as being representative of protein concentrations in erythrocytes from control subjects, and assuming  $5 \times 10^{12}$  /litre as the average erythrocyte concentration in

whole blood (Richmond and Davies, 1968), one can calculate that values reported here for ouabain binding in erythrocytes from control subjects are approximately half that reported by Erdmann and Hasse (1975). The discrepancy may be due in part to the assumption in these calculations that each erythrocyte has the same protein concentration.

The reduction in ouabain binding could explain the reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity seen in the erythrocyte membranes of unipolar depressed patients. However, it must be remembered that only four patients were investigated. This observation warrants further study. The measurement of ouabain binding during treatment and subsequent recovery would provide valuable additional information to the observations that erythrocyte membrane  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity either increases towards a normal value when patients are successfully treated with lithium (Hesketh *et al*, 1978), mianserin (Section III, part 3 of this thesis) and amitriptyline (Section III, part 3 of this thesis) or remains reduced when ECT is administered (see Section III, part 4).

It is interesting to speculate upon the cause of the reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity and ouabain binding. It is possible that the reduction in enzyme activity is due either to the removal of an 'enzyme activator' normally present or to the presence of an 'enzyme inhibitor'. It would be necessary to show that such a substance, as well as modulating  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity, also affected ouabain binding. Erdmann and Hasse (1975) have discussed in their paper which hormone or substance it is that naturally binds to the ouabain receptor and thereby regulates, or influences at least, the active transport of  $\text{Na}^+$  and  $\text{K}^+$ . If such a substance could be isolated, it would be interesting to see if its concentration in the bloodstream correlated with psychological ratings of depressed patients.



## 2. PLATELET MEMBRANE ADENYL CYCLASE ACTIVITIES

It has been proposed that a monoamine receptor abnormality occurs in certain depressed patients (Ashcroft, Eccleston, Murray, Glen, Crawford, Pullar, Shields, Walter, Blackburn, Connechan and Lonergan, 1972). One group that has tested this proposal examined platelet adenylyl cyclase responses to noradrenaline and prostaglandin  $E_1$  in 11 male depressed patients (4 unipolar depressed, 3 bipolar depressed, 4 with depressive neurosis; 10 of the 11 patients shown to be moderately depressed by Hamilton and Beck rating scales) and 8 male control subjects (Wang, Pandey, Mendels and Frazer, 1974a). Prostaglandin  $E_1$  stimulates the net synthesis of cAMP in platelets. Noradrenaline induces a decrease in this stimulation. Wang *et al* (1974a) selected the biochemical response to noradrenaline as suitable for examination as they considered the adrenergic receptor to be closely related to and possibly even part of adenylyl cyclase, the enzyme which catalyzes the conversion of ATP to cAMP. They adopted this strategy (examination of a response to a monoamine) in preference to measuring concentrations of monoamines or their metabolites in body fluids, studies which in the past had not produced unequivocal results (see Section I of this thesis). However, they appreciated that noradrenaline has an excitatory action on adenylyl cyclase in the brain, suggesting that platelets may not be a suitable system in which to evaluate responses to monoamines.

The study described in this thesis extended their observations (Wang *et al*, 1974a) by examining platelet adenylyl cyclase activity

in 62 male and female patients suffering from affective disorders (13 unipolar depressed, 8 unipolar well, 25 bipolar depressed, 8 bipolar manic and 8 bipolar well) and 26 control subjects, and by measuring the action of lithium on the activity of this enzyme. Fluoride was used in these studies to stimulate basal adenylyl cyclase activity (see Section II, part 8a of this thesis) not prostaglandin  $E_1$ . Both compounds are thought to act by increasing the affinity of the enzyme for magnesium (Wang, Pandey, Mendels and Frazer, 1974b).

The specific activity of adenylyl cyclase in platelet membrane preparations in this study was of a similar order to that described by Mendels and Frazer (1974). No change in adenylyl cyclase activity was found in any of the patient groups studied when compared with control subjects (Section III, part 1 of this thesis), which agrees with the observation of Wang *et al* (1974a) on basal adenylyl cyclase activity.

Investigation of the inhibitory action of noradrenaline by Wang *et al* (1974a) revealed no difference between patients and controls either.

Administration of lithium to either control subjects or certain groups of patients (Section III, part 1 of this thesis) revealed no modification of platelet adenylyl cyclase activity. This observation can be usefully compared to the work of Wang *et al* (1974b). Lithium *in vitro* was shown by this group to have an inhibitory action on platelet adenylyl cyclase activity which had been stimulated by prostaglandin  $E_1$ . The results presented in this thesis suggest that this inhibitory action of lithium is not irreversible.

### 3. 5-HT UPTAKE INTO PLATELETS AND WHOLE BLOOD 5-HT

A significant reduction was observed in the  $V_{\max}$  value (maximum transport rate under given experimental conditions) for 5-HT uptake into platelets from unipolar depressed (Section III, part 1 and 3) and bipolar depressed patients (Section III, part 1). The  $K_m$  value (Michaelis constant, considered to express the affinity of the substrate for the membrane carrier; the lower the  $K_m$ , the greater the affinity for the carrier) for this process was found not to differ from that in control subjects. These findings are similar to those reported by Hallstrom, Linford Rees, Pare, Trenchard and Turner (1976), Mulgirigama (1976) and Tuomisto and Tukianen (1976).

Values obtained for platelet 5-HT uptake (as characterized by  $V_{\max}$  and  $K_m$ ) in control subjects here, are in close agreement with those reported by Lingjaerde (1969, 1971) and Tuomisto and Tukianen (1976). However, estimates made by Coppen, Ghose, Swade and Wood (1978), Gordon and Olverman (1978) and Stahl and Meltzer (1978) for this process are rather different.

Disagreement exists among various workers as to the biochemical characteristics, and the kinetic and pharmacological properties of 5-HT transport in blood platelets (see Sneddon, 1973, for review). Sneddon has suggested that much of this variation could be attributed to varying and poorly controlled experimental conditions.

5-HT is thought to enter platelets by two mechanisms; an active transport process which predominates at low substrate concentrations, and simple diffusion which predominates at high substrate concentrations (Fuks, Lanman and Schanker, 1964). Thus, results obtained under conditions where diffusion could make a significant contribution to the total uptake should be treated cautiously.

Many workers use PRP, considering it more physiological than artificial media. However, plasma pH is difficult to control and the pH tends to rise throughout an incubation as the plasma loses carbon dioxide. Variations in 5-HT uptake may occur as a result.

If the sensitivity of the transport system to various inhibitors is being assessed using PRP, interpretation may be complicated by the adsorption of metabolic inhibitors and drugs by plasma proteins. This will result in a reduced effectiveness, especially at low concentrations of these compounds.

Divergent results may also occur if the experimental protocol does not allow a pre-incubation period, during which time inhibitors may act to establish their full effect. This is especially important when the actions of metabolic inhibitors are being considered.

In the studies reported in this thesis a five minute incubation time was chosen when 5-HT uptake into platelets was measured. When the initial rate of uptake is to be measured, as was the case in this study, the incubation time must be short enough to secure a nearly linear time-uptake plot. Several workers, using 5-HT concentrations of 1  $\mu\text{g/ml}$  and upwards, have found that the uptake is nearly linear for one hour (Born and Gillson, 1959; Hughes and Brodie, 1959; Weissbach and Redfield, 1960; Crawford, 1967). With the procedure used in the investigations reported in this thesis, however, the uptake was usually linear for 10 minutes. Therefore, an incubation time of 5 minutes was selected as being suitable. This is a rather longer incubation time than that employed by other workers (Gordon and Olverman, 1978) using low 5-HT concentrations. The difference is probably due to the lower rate of substrate depletion in this set of experiments, which existed as a result of the use of a more dilute suspension of platelets.

Uptake into platelets was measured using  $^3\text{H}$ -5-HT in a concentration range of 0.25-2  $\mu\text{M}$ . The investigations reported were concerned with active uptake only, and with substrate concentrations in this low range, passive uptake is thought to be virtually non-existent. This has been shown by the lack of any net uptake in the presence of imipramine in concentrations which do not increase outflux (Lingjaerde, 1971), and by the lack of any net uptake in the absence of chloride or sodium (Lingjaerde, 1969). Some workers measure 5-HT uptake at 0°-4°C and designate the amount of radioactively-labelled 5-HT found in the extracted platelets as that due to passive diffusion (Coppens *et al*, 1978). As the cell membrane constituents are likely to undergo considerable changes in physico-chemical properties in the transition from 37°-0°C it seems unlikely that such measurements will reflect passive diffusion at 37°C. As these workers used low 5-HT concentrations it seems likely that the value they obtained was actually a measure of 'trapped' and 'externally bound' 5-HT.

In the experiments reported in this thesis, an estimation was made of the small amount of  $^3\text{H}$ -5-HT trapped in pellets when platelets were isolated by centrifugation after incubation, and that bound onto the external face of the platelet plasma membrane during incubations. This binding may be to specific 5-HT receptors which play a part in platelet aggregation (Boullin, Molyneux and Roach, 1978) or it may be due to binding at non-specific sites. The 'blank' value was measured by estimating the amount of  $^3\text{H}$ -5-HT present after incubation for 5 minutes at 0° to 4°C. It is realized that such a measurement is only an approximation of the trapped and externally bound  $^3\text{H}$ -5-HT. The membrane changes occurring between 37° and 0°C may affect these parameters, as well. This 'blank' value could have been eliminated

by 'washing', i.e. by repeated resuspensions and recentrifugations. However, this procedure would have resulted in a loss of platelets and of platelet-granule bound 5-HT, and was therefore not used.

Uptake of 5-HT by platelets was measured in diluted PRP in the studies reported in this thesis. The pH was found to be stable during the course of 5 minute incubations which was probably due to the ratio of PRP added : total incubation volume, which was 1:40.

Although some consider it acceptable to treat transport kinetics analogously with enzyme kinetics (Stein, 1967), when 5-HT uptake into platelets is measured it is possible that several processes could be contributing to the observed effects. This may be important when interpreting the reduced  $V_{\max}$  value for 5-HT uptake into platelets from depressed patients.

The reduction observed could be due to a true reduction in 5-HT uptake at the plasma membrane (i.e. as a result of a decrease in the number of 5-HT 'carriers') or it could be due to an increase in 5-HT outflux as a result of, for example, increased intraplatelet 5-HT degradation by MAO or of decreased intragranular binding.

In the experiments reported here net uptake of 5-HT was measured. However, the true uptake would be obtained only when an estimate of outflux was made. Therefore, it is essential to know the rate of outflux which occurs in the experimental conditions used. It is often taken for granted that there is practically no outflux of 5-HT from platelets unless some releasing agent is present in the incubation medium. Pletscher, Bartholini and Da Prada (1966) found that when rabbit platelets were incubated in plasma or Tyrode solution, they lost only 1-2% of their 5-HT during 4-6 hours. Lingjaerde (1971) found that human platelets, preloaded with radio-actively labelled

5-HT, lost very little 5-HT when they were isolated and then resuspended and reincubated for one hour in phosphate buffer with NaCl and KCl added in suitable amounts. However, this does not constitute proof that there is no outflux going on during such an incubation, because the 5-HT leaking out of the platelets could be taken up again very rapidly, provided that the medium allows uptake of 5-HT. To measure outflux, it would be necessary to block the reuptake of 5-HT from the medium. This could be done by incubating the platelets in a medium lacking one of the ions necessary for active uptake, incubating in a medium containing a 5-HT uptake inhibitor (i.e. imipramine) or using platelets, preloaded with radioactively-labelled 5-HT, which would be subsequently resuspended in a medium containing a high concentration of unlabelled 5-HT. This would have the effect of blocking reuptake of labelled 5-HT by competition. None of these methods are ideal, because it is difficult to be sure that the composition of the medium does not influence the rate of outflux, besides blocking the reuptake. Studies performed by Lingjaerde (1971) suggest that in human platelets net uptake seems to be a fairly accurate measure of influx, the ratio of 5-HT influx to 5-HT outflux being about 12:1. However, it is quite possible that in platelets from patients with affective illness, the rate of outflux could be greater than that found in control subjects. It would be useful to estimate rates of outflux in platelets from patients who showed a reduction in platelet uptake.

Platelet MAO activity has been reported as being decreased in bipolar depressed patients. However, the activity may be increased in unipolar depressed patients (Buchsbaum, Landau and Murphy, 1973; Nies, Robinson and Harris, 1974). It is possible that the reduced



5-HT uptake observed in unipolar depressed patients may be explained by an increased MAO. However, platelets of all species tested so far (including man) metabolize 5-HT very slowly; more than 90% of the radioactivity in the platelet represents unmetabolized amine (Stacey, 1961; Pletscher, 1968; Solomon, Spirt and Abrams, 1970). Platelet MAO activity would have to be greatly increased in depressed patients before an effect was seen in 5-HT uptake.

It is thought that 5-HT is stored in platelet granules in 5-HT - ATP -  $Mg^{2+}$  micelles (Pletscher, Da Prada, Berneis and Tranzer, 1971). It is possible that a reduction in the ATP or  $Mg^{2+}$  content of platelet granules might produce a reduction in intraplatelet 5-HT binding. It is interesting to note that in Down's syndrome, a condition where a reduced platelet 5-HT uptake is also observed, a reduction in platelet ATP has been found (Boullin and O'Brien, 1971). However, to date there have been no investigations of ATP or magnesium content of platelets in affective illness. It is interesting that in depressed patients who showed reduced 5-HT uptake, whole blood 5-HT levels were not significantly different from control values (Section III, part 3 of this thesis). As platelets contain more than 99% of whole blood 5-HT (Pletscher, 1968), this suggests that there is no abnormality in the granule 5-HT binding capacity of these platelets. So, although these platelets do not take up 5-HT as fast as those from controls, they are still able to attain a normal 5-HT level.

It is possible that the defect seen in depression is due to a change in 5-HT transport across the platelet granule membrane. This process is often deemed to be unimportant in a consideration of overall 5-HT uptake. Recent work (Costa, Murphy and Reveille, 1977) has



shown that the dynamics of the human platelet amine uptake and storage system are complex, suggesting that this aspect might be important in an evaluation of total uptake.

In connection with the results obtained for 5-HT uptake it is interesting to reconsider work by McCoy *et al* (1974). These workers found  $\text{Na}^+ + \text{K}^+$ -ATPase activity to be low in platelets from patients with Down's syndrome. They considered this to be the cause of the reduced platelet 5-HT uptake observed in these patients. The transport of 5-HT into platelets is known to be an active process coupled to  $\text{Na}^+ + \text{K}^+$ -ATPase and absolutely dependent on sodium (Sneddon, 1969, 1971). McCoy *et al* (1974) proposed that the diminished  $\text{Na}^+ + \text{K}^+$ -ATPase activity in Down's syndrome would lead to elevated intraplatelet  $\text{Na}^+$  levels and diminished intraplatelet 5-HT levels. This could be explained in terms of the model proposed by Sneddon (1973) for 5-HT uptake into platelets. The proposed transport carrier is thought to have two binding sites, one for 5-HT and the other for monovalent cations. The carrier is assumed to migrate freely between the interfaces of the platelet plasma membrane. When one sodium ion and one molecule of 5-HT bind to the carrier it can migrate through the membrane so that both  $\text{Na}^+$  and 5-HT appear at the inner membrane face. In this position it is thought that intracellular  $\text{K}^+$  competes for the  $\text{Na}^+$  binding site, displacing  $\text{Na}^+$  and so reducing the affinity of the carrier for 5-HT, which would now dissociate from the carrier and be bound by the intracellular storage granules or metabolized by monoamine oxidase. The carrier could now freely migrate back across the membrane, where in an environment of high  $\text{Na}^+$  the cycle could start again. An essential feature of this proposed  $\text{Na}^+$ -dependent uptake process is that the intracellular  $\text{Na}^+$

concentration must be maintained at a low level relative to  $K^+$ . If the internal  $Na^+$  was allowed to rise, the  $Na^+ - K^+$  exchange process at the inner face of the membrane would be reduced. Thus, 5-HT would not be able to dissociate from the carrier as readily as before, and transport of 5-HT would thus be reduced. However,  $Na^+ + K^+ - ATPase$  activity was found to be normal in platelets from unipolar and bipolar depressed patients (Section III, part 1 of this thesis). It is therefore assumed that a low intracellular  $Na^+$  concentration was present in these platelets, and that this could not be the cause of the reduced 5-HT uptake observed.

It is interesting to speculate if the reduction observed in platelet 5-HT uptake parallels a change in 5-HT uptake in the central nervous system. It has been suggested that the blood platelet can serve as a model for transport processes in 5-hydroxytryptaminergic neurones in the CNS (Pletscher, 1968; Sneddon, 1973; Stahl, 1977). However, it has not been rigorously demonstrated that the accumulation of 5-HT in both tissues proceeds by analogous kinetic mechanisms which share the same pharmacological characteristics (Sneddon, 1973).

Smith, Hanson and Omenn (1978b) have tried to resolve these difficulties by examining 5-HT uptake into both platelets and synaptosomes using the same experimental protocol, i.e. a 2 minute incubation period and 5-HT concentrations in the range 0.025-1  $\mu M$ . They found that in five strains of mice and in rhesus macaques although the  $K_m$  values recorded are similar in platelets and synaptosomes, to the nearest order of magnitude, the two apparent  $K_m$  values for synaptosomes are both significantly different from the single apparent  $K_m$  value in platelets (Table IV.5). These workers also found that a specific 5-HT uptake inhibitor, Lilly compound 110140, inhibited 5-HT

TABLE IV.5:  $K_m$  values reported by Smith *et al* (1978b) for 5-HT uptake into platelets and synaptosomes

	Platelets	$K_m$ Synaptosomes
Mice (average for 5 strains)	154	55 and 610
Macaques	370	140 and 770

uptake much more effectively in platelets than in synaptosomes. Interpretation of their results is complicated by the fact that synaptosomes and platelets are isolated by different procedures. Smith *et al* (1978b) checked the action of anticoagulant on 5-HT-uptake in synaptosome preparations and found it to have no effect. However, it is interesting to note that they detected a small molecular weight factor in plasma, which was capable of inhibiting the high affinity uptake of 5-HT into synaptosomes. Apparently, the presence of this factor does not prevent the expression of the high affinity uptake of 5-HT into platelets. Their findings suggest that the transport systems for 5-HT into synaptosomes and platelets are in fact different.

Stahl and Meltzer (1978) have suggested that discrepancies in the reported values of 5-HT uptake into platelets and synaptosomes are due to incorrect kinetic analyses and differences in the concentration range of 5-HT studied. They suggest that an accurate measure of active saturatable 5-HT uptake can be made by subtracting the linear component (obtained by extrapolating to zero, uptake measured at high 5-HT concentration) from the total uptake. They consider this linear component to represent non-saturatable passive diffusion. Their results (Stahl and Meltzer, 1978; Figure 3) would

suggest that at fairly low 5-HT concentrations (1-2  $\mu\text{M}$ ) passive diffusion can contribute as much as 50-75% of total uptake. This finding disagrees with that of other workers (Lingjaerde, 1971; Le Fur and Uzan, 1977; Gordon and Olverman, 1978). Stahl and Meltzer (1978) quote IC50 values for inhibition of 5-HT uptake into platelets by tricyclic antidepressants which they estimated from log probit plots constructed from six different drug concentrations. It would be interesting to know if these workers achieved 100% inhibition at low 5-HT concentrations (i.e. 1-2  $\mu\text{M}$ ) using a concentration of tricyclic which affected uptake only. If they did, it would suggest that either their experimental procedure or their method of calculating active uptake is incorrect.

It has been shown that a deficiency in platelet 5-HT level observed in Fawn-Hooded rats is not reflected by a similar reduction in 5-HT in the central nervous system (Joseph, 1978). This may be relevant to inferences concerning the central nervous system, drawn from the observation that platelet 5-HT uptake is reduced in depressed patients. However, it is not known if a reduction in 5-HT uptake exists in the platelets from Fawn-Hooded rats. These platelets are known to have a greatly reduced ATP content, so it is probable that the platelet abnormality seen in these rats is more akin to that of 'storage pool disease' in humans, and thus does not shed any light on aberrations present in the brains of patients with affective disorders.

It must be remembered that even if it could be shown conclusively that platelets and synaptosomes used the same type of 5-HT carrier, changes occurring in platelets in affective illness could still be due to the presence of a substance in the blood which might not have

access to the central nervous system. It would be useful to know if platelet and brain 5-HT transport systems are expressed by the same or different genes, and if they are expressions of the same gene, to what extent the two mechanisms are differentially modified in the two tissues.

The action of lithium, mianserin and amitriptyline therapy was investigated in unipolar depressive patients to see if these drugs modified the reduced platelet 5-HT uptake. Lithium administration and recovery did not affect 5-HT uptake in any of the patient groups or controls studied (Section III, part 1), except for a tendency to increase the rate of uptake in the unipolar and bipolar depressed group. Murphy, Colburn, Davis and Bunney (1969, 1970) found that lithium therapy stimulated uptake of monoamines into platelets from depressed patients. It is possible that if a larger number of patients taking lithium had been investigated it might have been possible to observe the definite effect of lithium reported by this group (Murphy *et al*, 1969, 1970). Control subjects were not investigated (Murphy *et al*, 1969, 1970) so it is not known if platelet 5-HT uptake was less than normal before therapy commenced. It is also unclear from their paper, if after two weeks of lithium administration, the patients investigated had recovered. Thus, it is not known if these workers were investigating true lithium actions or if recovery effects were being observed. This group also investigated the *in vitro* action of lithium and found that 5-HT uptake into platelets from control subjects was not increased. The results presented in this thesis are in agreement with this finding.

The lithium concentrations investigated *in vitro* were in the range expected in plasma when the drug is given therapeutically.

It is known that lithium binding to plasma proteins is negligible (Gilman , Goodman and Gilman, 1980). It is not known, however, if the difference between lithium *in vivo* and *in vitro* is due to a difference in intraplatelet lithium concentration. It would be useful to measure this concentration in platelets from control subjects after a ten minute incubation period with the drug, and make a comparison with values obtained in platelets from patients receiving lithium. It is possible that the difference is due to differences in the time the platelets are exposed to the drug. Imandt, Genders, Wessels and Haanen (1977) have shown that lithium requires 90 to 120 minutes to reach an equilibrium concentration across platelet membranes.

It is possible that if lithium *in vivo* truly stimulates 5-HT uptake it may be producing the effect indirectly as a result of the recovery process or indirectly via some other mechanism. As 5-HT uptake is not stimulated in control subjects taking lithium it seems unlikely that it is as a result of some unidentified indirect activation. Whether the change observed is due to a recovery effect could be tested by comparing lithium-treated patients who recovered with lithium-treated patients who remained ill. This could not be carried out in this study as suitable patients were not available.

Results presented in Section III, part 3 showed that mianserin and amitriptyline administration and subsequent recovery had marked effects on platelet 5-HT uptake. However, it was shown that mianserin produces quite a different effect from amitriptyline. Although all patients in both groups recovered, it can be seen that the  $V_{max}$  values in the mianserin treated group approached control values while in the amitriptyline treated group  $V_{max}$  values were reduced still further, with a concomitant increase in  $K_m$  values. It would have

been useful to investigate mianserin and amitriptyline treated patients who failed to recover. It would then have been possible to dissociate drug effects from 'recovery from illness' effects.

However, some measure of this is available from an examination of the *in vitro* action of these drugs on platelets from control subjects. The *in vitro* action of amitriptyline was shown to be identical to its *in vivo* action. The concentration of amitriptyline used for *in vitro* studies on platelet 5-HT uptake was between 50-150 ng/ml (160-480  $\mu$ M). It is not known what the plasma concentration of amitriptyline was in the patients receiving the drug in this study. Coppen (1976) estimated amitriptyline plasma levels to be in the order of 25-200 ng/ml (80-645  $\mu$ M) when patients received 150 mg of the drug per day. It would appear that the concentration of amitriptyline required to appreciably inhibit 5-HT uptake is considerably less than that present therapeutically. Work by Todrick and Tait (1969) established that the ID<sub>50</sub> for amitriptyline was 5.3  $\mu$ M. However, it is known that plasma binding of tricyclic antidepressants occurs (Campbell and Todrick, 1970). This binding was calculated by these workers as reducing the effective concentration by 73-92%. Taking these estimates into account it is still probable that the 'free' concentration in the plasma (6-174  $\mu$ M) is much higher than that required to inhibit 5-HT uptake.

The *in vitro* action of mianserin, which is entirely different to its effect *in vivo*, is similar to the action of amitriptyline. Concentrations of mianserin chosen to investigate the *in vitro* action of the drug (25-75 ng/ml; 84-252  $\mu$ M) are in the range found in the plasma of patients receiving 60 mg of the drug per day (Coppen, Gupta, Montgomery, Ghose, Bailey, Burns and de Ridder, 1976; Montgomery,



McAuley and Montgomery, 1978; Perry, Fitzsimmons, Shapiro and Irwin, 1978). The difference between *in vivo* and *in vitro* effects might be explained by the proposal that a metabolite of mianserin is producing the effects seen *in vivo* (Coppen *et al*, 1978). It would have been more informative if an examination of the *in vitro* nature of these drugs on platelets from ill untreated patients had been made. Platelets from control subjects differ from those of ill patients, and could react in a different manner to these drugs.

Coppen *et al* (1978) investigated the *in vivo* and *in vitro* actions of mianserin on 5-HT uptake into platelets from depressed patients. They found that mianserin administration *in vivo* resulted in an increase in  $V_{max}$  values while the opposite effect occurred *in vitro*. They found that mianserin caused  $K_m$  values to increase both *in vivo* and *in vitro*. It is difficult to explain why  $K_m$  values increase in the Coppen *et al* (1978) study after *in vivo* administration while no such change was observed in the study reported in this thesis. Coppen *et al* (1978) measured plasma mianserin levels and knew for sure that their patients had taken their medication. However, if a mianserin metabolite is responsible for the effects observed, this measurement is irrelevant unless mianserin is metabolized in the same manner by all patients. It was not known in the study reported in this thesis if patients ingested their medication. If the patients failed "to comply" but still recovered, it is possible that some effects of the drug may have been missed.

A consideration of the uptake kinetics after exposure of these drugs to platelets suggests that *in vitro* both amitriptyline and mianserin act as non-competitive inhibitors. This implies that the two drugs effect their inhibition not by modifying binding of 5-HT



to the carrier system, but by reducing movement of the carrier through the membrane (Wold, 1971).

Kinetic studies of the effects of tricyclic antidepressants have usually shown the inhibition to be competitive in nature (Stacey, 1961; Yates, Todrick and Tait, 1964; Tuomisto, 1974; Gordon and Olverman, 1978). This implies a direct action on the 5-HT binding site, with inhibition being reduced at higher substrate concentrations.

However, Le Fur and Uzan (1977) considered imipramine, clomipramine, desipramine and amitriptyline to be uncompetitive inhibitors. It is probable that these authors have made a typographical error. Their plots of  $1/v$  against  $1/s$  show non-competitive inhibition (change in  $V_{max}$ , no change in  $K_m$ ).

Coppen *et al* (1978) obtained the same *in vitro* effect for mianserin as described here, but described the inhibition as competitive.

It is interesting that when amitriptyline was administered whole blood 5-HT levels were shown to decrease to a very low level, as the  $V_{max}$  values decreased. As the decreased  $V_{max}$  values found in untreated ill patients do not occur concomitantly with low whole blood 5-HT levels it would appear that amitriptyline *in vivo* has actions unrelated to its blockade of 5-HT uptake. It is possible that at the concentrations present in plasma it causes release of 5-HT from platelets. It is also possible that it interferes with the production of 5-HT by the enterochromaffin cells in the gut, thereby reducing the amount of 5-HT available for entry into platelets. Platelet bound 5-HT is thought to be decreased by 3-30% during passage through the lungs (Crawford, 1965). It is possible that in

amitriptyline treated patients this percentage is greatly increased. Platelet-bound 5-HT has been reported as having a shorter half-life in the body than the platelets themselves, and that this is caused by an exchange of platelet 5-HT with 5-HT depots elsewhere in the body (Marcus and Zucker, 1965). Amitriptyline may interfere with these processes. As the decrease in whole blood 5-HT occurs over a period of two weeks, it is quite likely that amitriptyline has either an action at the megakaryocyte level directly and is affecting platelets as they are being manufactured or that young platelets are more susceptible to changes in other systems that may be affected by the drug.

It is possible that the reduction in whole blood 5-HT is due merely to a change in the ratio of influx: outflux of 5-HT. After amitriptyline administration,  $V_{\max}$  values are reduced even further below those found in ill untreated patients. It is possible that if in this situation the influx (i.e. uptake) is less than the outflux, platelet 5-HT levels would be expected to drop.

Work by other groups on platelet 5-HT uptake and the action of tricyclic antidepressants, have revealed rather different effects.

Tuomisto and Tukianen (1976) carried out an investigation on 5-HT uptake and the effect of imipramine on the uptake in blood platelets obtained from depressed patients. They found that after four weeks treatment the  $V_{\max}$  for the process increased

towards normal values, whereas the  $K_m$  was increased above normal. In vitro, addition of the drug to the platelet suspension (in a concentration equivalent to that expected in the plasma) led to  $K_m$  values being increased without a concomitant  $V_{max}$  change. They concluded that the  $V_{max}$  change observed in treated patients was associated with recovery from depression and was not a drug effect. This is not a valid conclusion. It is quite possible that the administered drug acts at some unknown site in the body, which can then subsequently exert an influence on platelet 5-HT uptake. This process may be totally unrelated to the recovery of the patient from depression.

In an earlier study by Murphy *et al* (1969), imipramine therapy had also been found to cause an increase in  $K_m$  values, with a reduced initial rate of uptake but no change in  $V_{max}$  values. However, not all of the patients studied by this group, recovered during the investigation period. In keeping with the observation of Murphy *et al* (1969), Marshall, Stirling, Tait and Todrick (1960) found that imipramine administration caused an 84% fall in platelet 5-HT levels. This suggests that imipramine was acting as a competitive inhibitor, and it was present in sufficient concentration to prevent most of the 5-HT, to which the platelets were exposed in the body, from being taken up.

It would appear from results presented in this thesis, that an increase in  $V_{max}$  does not necessarily occur as a patient recovers from depression. However, it is not known whether this

is due to the different therapies used. An examination of platelets from depressed patients being treated successfully by behavioural therapy would be useful in differentiating recovery from drug effects.

#### 4. VARIATION IN SERIAL PREPARATIONS OF PLATELETS, ERYTHROCYTES AND WHOLE BLOOD 5-HT

There was considerable variation in ATPase activities in platelet and erythrocyte membranes prepared from a single control subject on successive occasions. This variation was especially obvious in  $\text{Na}^+ + \text{K}^+$ -ATPase activities measured at intervals during a menstrual cycle. Substantial changes in activity occurred in some cases in the course of a week. A standard ATPase, assayed together with the membrane preparations, showed little variation in enzyme activity so providing evidence that the variation in membrane ATPase activities was not due to methodological error in the ATPase assay. The variation in activity must therefore have been due either to *in vivo* variation, to variation in the sample of blood cells taken or to variation produced during the preparation of the membranes.

Sampling variation could have occurred if successive samples contained different populations of platelets and erythrocytes in respect to cell age. It has been suggested that the age distribution of erythrocytes could be a cause of variation in membrane preparations (Hanahan, 1973). However, Cohen, Ekholm, Luthra and Hanahan (1975) were unable to show any consistent relationship between erythrocyte cell age as judged by cell density and ATPase activities in freeze-thawed cells. Other workers have shown  $\text{Ca}^{2+}$ -ATPase activity in erythrocyte membrane preparations to depend on cell age and in addition have found  $\text{Mg}^{2+}$ -ATPase activity to be increased in people with high (10-30%) reticulocyte counts (Feig and Guidotti, 1974). It is thought that platelets undergo considerable changes as they age in the circulation (Karpatkin and Amorosi, 1977). However, it is not known if different levels of ATPase activity are associated with

'young' and 'old' platelets. As the life of the mature human erythrocyte is known to be approximately 120 days (Harris and Kellermeyer, 1970), it seems unlikely that changes observed in the course of a week are due to changes in the population of red blood cells. The platelet is thought to have a life span of 8-11 days (Richmond and Davies, 1968), so in this case, it is possible that variation occurring over a short time could be due to cell turnover.

Variation in specific enzyme activity in the erythrocyte could have arisen from variation in the enzyme or protein content of the membrane. Hanahan and Ekholm (1972) showed that under preparative conditions similar to those used in these experiments,  $Mg^{2+}$ -ATPase and  $Ca^{2+}$ -ATPase activity was lost during preparation but there was no loss of  $Na^{+}+K^{+}$ -ATPase activity. Variation in such loss could lead to variation in enzyme activity in the final preparation.

Using identical preparative conditions, Hesketh and Reading (1978) investigated the cause of variation seen in ATPase activity of erythrocyte membranes. They found on a given day membrane preparations from a single haemolysate were reproducible in terms of protein yield and ATPase activities. This suggests that the centrifugation steps employed in the membrane preparation were reproducible. Using an angle head rotor, membrane yield was found to be drastically increased. However, they found that the specific activities of the ATPases were unchanged, suggesting that the membrane suspensions during preparation were not heterogeneous. Membrane ATPase activities determined on successive occasions and expressed per litre of original cells and per milligramme of protein were shown to be definitely correlated. This observation implies that variation between individuals is not due to either variation in membrane yield

in terms of protein or in terms of enzyme. This conclusion is supported by the lack of correlation between protein yield and ATPase specific activities. The only variable step in their preparation was the haemolysis. Cells were lysed in a constant volume-to-volume ratio, but the haematocrit of the cell suspension was shown to vary. However, this is probably unimportant as the cells were always suspended in iso-osmolar NaCl.

If the study of Hesketh and Reading (1978) can be applied to results given in this thesis, and it surely can as identical procedures were employed, it suggests that no methodological explanation exists to explain the observed variation in ATPase activities in successive samples. Hesketh and Reading (1978) suggest that the variation observed might be due to enzyme activation. Activators of  $\text{Ca}^{2+}$ -ATPase are known to exist (Jarrett and Penniston, 1978), although their presence might not be expected after the extensive washing procedures used in the membrane preparation. Results presented in Section III, part 4 of this thesis, suggest that changes in erythrocyte membrane ATPase activity can occur within minutes. Such a rapid change could be explained in terms of fluctuating plasma levels of activators.

Variation (up to 50%) in ATPase activity in platelet preparations has been reported by Moake *et al* (1970). They did not offer an explanation for this, but did state that there were no differences in basic properties of the preparations or in the effects of added inhibitors.

Results presented illustrate that serial samples can show considerable variation in ATPase specific activities, suggesting that interpretations made on a single preparation should be treated



with caution. Considerations of this nature are important in studies when enzyme activities are compared between different individuals, even although the value may be absolute for any particular individual at any particular time.

A large variation was seen in whole blood 5-HT levels in a single subject, especially during the course of a menstrual cycle. This disagrees with the findings of Ashcroft *et al* (1964). These workers found that although there was a large variation in the blood 5-HT concentration between individuals, whole blood 5-HT proved remarkably constant in a given individual on successive occasions.

Whole blood 5-HT is known to be susceptible to changes in diet (Ashcroft *et al*, 1964). It is conceivable that such an influence was responsible for the variations observed.

Wirz-Justice, Feer and Richter (1977a) measured platelet 5-HT at monthly intervals in subjects for an entire year. Fluctuations of greater than 300% were found, 5-HT levels being highest in late autumn and spring.

Platelet 5-HT has been shown to have a diurnal rhythmicity (Sauerbier and von Mayersbach, 1976; Wirz-Justice, Lichtsteiner and Feer, 1977b). No significant differences in whole blood 5-HT between men and women were seen in these studies, although changes related to the menstrual cycle were observed, but the sample was too small to be significant.

It would appear that data collected from individuals at a few time intervals requires a great deal of caution in interpretation, as allowance must be made for circadian, hormonal and seasonal determinants. Swiss researchers have suggested that this caution must be extended to studies involving patients with affective disorders.



Wirz-Justice and Pühringer (1978a,b) investigated manic-depressed patients to see if variations in whole blood 5-HT levels occurred. They found that the diurnal rhythm (but not the absolute level) of 5-HT was different in unipolar depressed patients compared to control subjects. Patients with bipolar illnesses were shown to have a similar diurnal rhythm as controls. However, these patients had higher absolute levels of whole blood 5-HT which was shown to be independent of clinical state.

Wirz-Justice and Pühringer (1978a) considered that the altered diurnal rhythm in platelet 5-HT did not appear to be related to changes in either platelet MAO activity or 5-HT uptake. Platelet MAO activity did not show a diurnal rhythm and there was no difference in 5-HT uptake when unipolar depressed patients were compared with controls. They observed a positive correlation between 5-HT uptake and platelet 5-HT content when measured at 8am, in both controls and unipolar depressed patients. However, they did not estimate this correlation in measurements made at 4pm, at a time when the greatest difference in platelet 5-HT is seen.

Wirz-Justice and Pühringer (1978a) feel that their observations (altered rhythms but no change in concentrations of two peripheral serotonergic parameters) are of interest 'in the light of the concept of a desynchronization phenomenon underlying depressive states.' They feel that the changing rhythm seen in platelet 5-HT parallels similar changes in the CNS. However, they can provide no evidence for this.

The variation in 5-HT uptake (as measured by  $V_{\max}$  values) into platelets from a single subject (see this thesis Section III part 6 (g) (iii)) was seen to be greatest during the course of a menstrual cycle. It is possible that hormonal changes are responsible for this variation.

No attempt was made to estimate the methodological error which occurred when 5-HT uptake was measured, other than carrying out estimations in duplicate. A suitable standard, similar to the kind used when the ATPase activity estimations were checked, was obviously not available. It is possible that the method used for stopping 5-HT uptake into platelets (transfer of tubes from 37°C to 0 - 4°C) was not consistently reproducible. Other methods are available. Costa and Murphy (1975) have investigated the use of fixatives, and have shown a 1.5% solution of formaldehyde to be satisfactory in stopping uptake and release very quickly.

## 5. CONCLUDING REMARKS

The first aim of this project (see page 61-62 of this thesis) was to conduct a large scale study in one establishment in an attempt to reduce variation in psychiatric assessment and laboratory procedures. Results reported in this thesis were obtained from 153 control, 77 unipolar depressed, 8 unipolar well, 29 bipolar depressed, 23 bipolar manic and 8 bipolar well subjects; a not inconsiderable total of 298 individuals.

It had also been hoped that by using 'finer' psychiatric classification than many previous studies had employed, valuable information would have been obtained. Patients in this study were rated according to the course of their illness (i.e. unipolar or bipolar), but unfortunately, except in the ECT study (Section 111, part 4), no measure of the degree of severity of their illness was made. This is a major limitation. In the studies reported in Section 111 parts 1 and 2, patients had been drug-free at least 2 weeks. No HRS values were obtained for these patients. It is probable that they were not so severely ill as some other patients studied, as they could be 'maintained' for this length of time without medication.

All patients receiving treatment recovered. It was therefore not possible to dissociate recovery effects from therapy effects, in the way that Hesketh *et al* (1978) had done. However, it is pertinent to consider that patients who fail to recover when treated in a certain manner, may well belong to a different 'sub-class' of the total population of depressed patients. Plasma levels of mianserin and amitriptyline were unfortunately not measured in patients receiving these drugs. It is therefore not known if the

patients ingested their prescribed medication.

Red blood cells and platelets from patients suffering from affective disorders were found to be different, in several respects, from those from control subjects.

The reduction in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in red blood cells, which occurred in both unipolar and bipolar depression, was not observed in platelets. Caution should thus be exercised when extrapolating changes seen in the periphery (i.e. change in one cell in periphery = change in all cells) to changes expected in the CNS.

After some treatments the reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity in erythrocytes increased to normal. However, no such change occurred after ECT. From results presented here, it is not possible to say if this reflects a difference between patients who require ECT and those who are satisfactorily treated with drugs.

The reduced  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity was found to be associated in 4 patients with a reduction in ouabain binding (and therefore in  $\text{Na}^+$  pump sites). This observation coupled with that of rapid changes seen in ATPase activity would suggest looking for an endogenous substance capable of modifying both enzyme activity and binding sites. It would then be useful to see if mood was positively correlated with the plasma concentration of this substance. Work very recently published (Naylor, Smith, Dick, Dick, McHarg and Chambers, 1980) suggests that a reduction in ouabain binding does not occur in affective illness. These workers investigated both depressed and manic patients. Although they did not observe any significant difference in  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity when ill patients were compared with recovered, they did observe a decrease in the ATPase activity per pump site. They suggest as well that

some endogenous substance must be influencing the  $\text{Na}^+$  pump, and have suggested vanadium as a likely candidate. They have shown that plasma vanadium is increased in manic-depressives compared to controls (Dick, Dick and Naylor, 1981) and that a low vanadium diet supplemented with ascorbic acid and EDTA has beneficial effects on manic-depressed patients (Naylor and Smith, 1981). It is known that vanadium can inhibit  $\text{Na}^+ + \text{K}^+ - \text{ATPase}$  activity (Cantley *et al* , 1978). However, values obtained in both patients and controls for plasma vanadium are well within the normal range. When patients improved on a low vanadium diet no estimate was made of their plasma vanadium levels. Also no details of the low vanadium diet were given. It is difficult to draw any conclusions from this work, on the importance of vanadium in the aetiology of affective illness.

Platelets from depressed patients were shown to have a reduced  $V_{\text{max}}$  for the uptake process. In mianserin-treated patients this returns to control values, whereas in amitriptyline-treated patients the values fall still further. As all patients recovered it is not possible to say what effects are due to treatment. It would be interesting to try and find out which controlling factor of 5-HT uptake sites is deranged in these patients, and see if that control varied with changing mood.

The work presented in this thesis has revealed that there are several interesting abnormalities in the blood cells of patients with affective illness. It is imperative that the cause of these changes be found. It is possible that this may reveal the cause of the illness i.e. high levels of vanadium may indeed cause affective illness. Over the last 30 years psychiatrists have searched for the key to affective illness. No treatment, which has been shown to be unequivocally successful, has been introduced as a result of all this expensive effort. It would seem important not to waste any more time and money.

## BIBLIOGRAPHY

- ABDULLAH, Y.H. and HAMADAH, K. (1970). 3'5' - Cyclic adenosine monophosphate in depression and mania. *Lancet*, i, 378-381.
- ABRAMS, R., ESSMAN, W.B., TAYLOR, M.A. and FINK, M. (1976). Concentration of 5-hydroxyindoleacetic acid, homovanillic acid, and tryptophan in the cerebrospinal fluid of depressed patients before and after ECT. *Biol. Psychiatry*, 11, 85-90.
- AKAGAWA, K., WATANABE, M. and TSUKADA, Y. (1980). Activity of erythrocyte Na,K - ATPase activity in manic patients. *J. Neurochem.*, 35, 258-260.
- ASHCROFT, G.W., BLACKBURN, I.M., ECCLESTON, D., GLEN, A.I.M., HARTLEY, W., KINLOCH, N.E., LONERGAN, M., MURRAY, L.G. and PULLAR, I.A. (1973a). Changes on recovery in the concentrations of tryptophan and the biogenic amine metabolites in the cerebrospinal fluid of patients with affective illness. *Psychol. Med.*, 3, 319-325.
- ASHCROFT, G.W., CRAWFORD, T.B.B., BINNS, J.K. and MACDOUGALL, E.J. (1964). Estimation of 5-hydroxytryptamine in human blood. *Clin. Chim. Acta*, 9, 364-369.
- ASHCROFT, G.W., CRAWFORD, T.B.B., CUNDALL, R.L., DAVIDSON, D.L., DOBSON, J., DOW, R.C., ECCLESTON, D., LOOSE, R.W. and PULLAR, I.A. (1973b). 5-Hydroxytryptamine metabolism in affective illness: The effect of tryptophan administration. *Psychol. Med.*, 3, 326-332.
- ASHCROFT, G.W., CRAWFORD, T.B.B., ECCLESTON, D., SHARMAN, D.F., MACDOUGALL, E.J., STANTON, J.B. and BINNS, J.K. (1966). 5-Hydroxyindole compounds in the cerebrospinal fluid of patients with psychiatric or neurological diseases. *Lancet*, ii, 1049-1052.
- ASHCROFT, G.W., DOW, R.C. and MOIR, A.T.B. (1968). The active transport of 5-hydroxyindole-3-acetic acid and 3-methoxy-4-hydroxyphenylacetic acid from a recirculatory perfusion system of the cerebral ventricles of the unanaesthetized dog. *J. Physiol. (Lond.)*, 199, 397-424.
- ASHCROFT, G.W., ECCLESTON, D., MURRAY, L.G., GLEN, A.I.M., CRAWFORD, T.B.B., PULLAR, I.A., SHIELDS, P.J., WALTER, D.S., BLACKBURN, I.M., CONNECHAN, J. and LONERGAN, M. (1972). Modified amine hypothesis for the aetiology of affective illness. *Lancet*, ii, 573-577.
- ASHCROFT, G.W. and GLEN, A.I.M. (1974). Mood and neuronal functions: a modified amine hypothesis for the etiology of affective illness. *Adv. Biochem. Psychopharmacol.*, 11, 335-339.
- ASHCROFT, G.W. and SHARMAN, D.F. (1960). 5-Hydroxyindoles in human cerebrospinal fluids. *Nature*, 186, 1050-1051.
- BAER, L., DURELL, J., BUNNEY, W.E., Jr., LEVY, B.S., MURPHY, D.L., GREENSPAN, K. and CARDON, P.V. (1970a). Sodium balance and distribution in lithium carbonate therapy. *Arch. Gen. Psychiatry*, 22, 40-44.

- BAER, L., PLATMAN, S.R. and FIEVE, R.R. (1970b). The role of electrolytes in affective disorders. *Arch. Gen. Psychiatry*, 22, 108-113.
- BAKER, E.F. (1971). Sodium transfer to cerebrospinal fluid in functional psychiatric illness. *Can. Psychiatr. Assoc. J.*, 16, 167-170.
- BALDESSARINI, R.J. and LIPINSKI, J.G. (1975). Lithium salts 1970-1975. *Ann. Intern. Med.*, 83, 527-533.
- BARBER, A.J. and JAMIESON, G.A. (1970). Isolation and characterization of plasma membranes from human blood platelets. *J. Biol. Chem.*, 245, 6357-6365.
- BARKAI, A., GLUSMAN, M. and RAPPORT, M.M. (1972). Serotonin turnover in the intact cat brain. *J. Pharmacol. Exp. Ther.*, 181, 28-35.
- BARTHOLINI, G. and PLETSCHER, A. (1964). Two types of 5-hydroxytryptamine release from isolated blood platelets. *Experientia*, 20, 376-378.
- BENKERT, O., RENZ, A., MARANO, C. and MATUSSEK, N. (1971). Altered tyrosine daytime plasma levels in endogenous depressive patients. *Arch. Gen. Psychiatry*, 25, 359-363.
- BERG, G.R. and GLINSMANN, W.H. (1970). Cyclic AMP in depression and mania. *Lancet*, i, 834.
- BETTEX-GALLAND, M. and LÜSCHER, E.F. (1961). Thrombosthenin - a contractile protein from thrombocytes. Its extraction from human blood platelets and some of its properties. *Biochim. Biophys. Acta*, 49, 536-547.
- BHARGAVA, N., MATTHIJSSEN, R. and SCHÖNBAUM, E. (1977). Some effects of Org GC 94, a potential anti-migraine drug, on human platelets. *Eur. J. Pharmacol.* 42, 241-245.
- BILLE, P.E., JENSEN, M.K., KAALUND-JENSEN, J.P. and PAULSEN, J.C. (1975). Studies on the haematologic and cytogenetic effect of lithium. *Acta Med. Scand.*, 198, 281-286.
- BIRCH, N.J. (1973). The role of magnesium and calcium in the pharmacology of lithium. *Biol. Psychiatry*, 7, 269-272.
- BOARDMAN, L.J., HUME, S.P., LAMB, J.F. and POLSON, J. (1975). Effect of growth in lithium on ouabain binding, Na-K-ATPase and Na and K transport in Hela cells. *J. Physiol. (Lond.)*, 244, 677-682.



- BODEMAN, H. and PASSOW, H. (1972). Factors controlling the resealing of the membrane of human erythrocyte ghosts after hypotonic hemolysis. *J. Membr. Biol.*, 8, 1-26.
- BOISSIER, J.R., GODEFROY, F., SOUBRIE, P., THIEBOT, M.H. and WEIL-FUGAZZA, J. (1976). Platelet uptake of [ $^{14}\text{C}$ ]-5-hydroxytryptamine in 'emotional' and 'non-emotional' rats. *Br. J. Pharmacol.*, 58, 454P-455P.
- BOND, P.A., JENNER, F.A. and SAMPSON, G.A. (1972). Daily variations of the urine content of 3-methoxy-4-hydroxyphenylglycol in two manic depressive patients. *Psychol. Med.*, 2, 81-85.
- BORN, G.V.R. and GILLSON, R.E. (1959). Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J. Physiol. (Lond.)*, 146, 472-491.
- BOULLIN, D.J., MOLYNEUX, D. and ROACH, B. (1978). The binding of haloperidol to human blood platelets and interactions with 5-hydroxytryptamine and dopamine. *Br. J. Pharmacol.*, 63, 561-566.
- BOULLIN, D.J. and O'BRIEN, R.A. (1971). Abnormalities of 5-hydroxytryptamine uptake and binding by blood platelets from children with Down's syndrome. *J. Physiol. (Lond.)*, 212, 287-297.
- BOURNE, H.R., BUNNEY, W.E., Jr., COLBURN, R.W., DAVIS, J.M., DAVIS, J.N., SHAW, D.M. and COPPEN, A.J. (1968). Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in hindbrain of suicidal patients. *Lancet*, ii, 805-808.
- BOWERS, M.B., Jr. (1972). Clinical measurements of central dopamine and 5-hydroxytryptamine metabolism: Reliability and interpretation of cerebrospinal fluid acid monoamine metabolite measures. *Neuropharmacology*, 11, 101-111.
- BOWERS, M.B., Jr. (1974). Lumbar CSF 5-hydroxyindoleacetic acid and homovanillic acid in affective syndromes. *J. Nerv. Ment. Dis.*, 158, 325-330.
- BOWERS, M.B., Jr., and GERBODE, F. (1968a). Relationship of monoamine metabolites in human cerebrospinal fluid to age. *Nature*, 219, 1256-1257.
- BOWERS, M.B., Jr., and GERBODE, F. (1968b). CSF 5-HIAA. Effects of probenecid and parachlorophenylalanine. *Life Sci.*, 7, 773-776.
- BOWERS, M.B., Jr., HENINGER, G.R. and GERBODE, F. (1969). Cerebrospinal fluid 5-hydroxyindoleacetic acid and homovanillic acid in psychiatric patients. *Int. J. Neuropharmacol.*, 8, 255-262.
- BRAMLEY, T.A., COLEMAN, R. and FINEAN, J.B. (1971). Chemical, enzymological and permeability properties of human erythrocyte ghosts prepared by hypotonic lysis in media of different osmolarities. *Biochim. Biophys. Acta*, 241, 752-769.

- BRIDGES, P.K. (1978). New antidepressant drugs. *J. Pharmacotherapy*, 1, 12-19.
- BUCHSBAUM, M., LANDAU, S. and MURPHY, D. (1973). Average evoked response in bipolar and unipolar affective disorders: relationship to sex, age of onset, and monoamine oxidase. *Biol. Psychiatry*, 7, 199-212.
- BUNNEY, W.E., Jr., BRODIE, H.K.H., MURPHY, D.L. and GOODWIN, F.K. (1971). Studies of alpha-methyl-para-tyrosine, L-dopa and L-tryptophan in depression and mania. *Am. J. Psychiatry*, 127, 872-881.
- BUNNEY, W.E., Jr., and DAVIS, J.M. (1965). Norepinephrine in depressive reactions: A review. *Arch. Gen. Psychiatry*, 13, 483-494.
- BUNNEY, W.E., Jr., MURPHY, D.L. and GOODWIN, F.K. (1970). The switch process from depression to mania: Relationship to drugs which alter brain amines. *Lancet*, i, 1022-1027.
- CADE, J.F.J. (1949). Lithium salts in the treatment of psychotic excitement. *Med. J. Aust.*, 2, 349-352.
- CALNE, D.B., KAROUM, F., RUTHVEN, C.R.J. and SANDLER, M. (1969). The metabolism of orally administered L-DOPA in Parkinsonism. *Br. J. Pharmacol.*, 37, 57-68.
- CAMPANINI, T., CATALANO, A., DERISIO, C. and MARDIGHIAN, G. (1970). Vanilmandelic aciduria in the different clinical phases of manic depressive psychoses. *Br. J. Psychiatry*, 116, 435-436.
- CAMPBELL, I.C. and TODRICK, A. (1970). Plasma protein binding of tricyclic antidepressive drugs. *J. Pharm. Pharmacol.*, 22, 226-227.
- CANTLEY, L.C., Jr., RESH, M.D. and GUIDOTTI, G. (1978). Vanadate inhibits the red cell ( $\text{Na}^+$ ,  $\text{K}^+$ ) ATPase from the cytoplasmic side. *Nature*, 6, 552-554.
- CARMAN, J.S., POST, R.M., GOODWIN, F.K. and BUNNEY, W.E. Jr. (1977). Calcium and electroconvulsive therapy of severe depressive illness. *Biol. Psychiatry*, 12, 5-17.
- CARNEY, M.W.P., SHEFFIELD, B.F. and SEBASTIAN, J. (1973). Serum magnesium, diagnosis, ECT and season. *Br. J. Psychiatry*, 122, 427-429.
- CARROLL, B.J. (1972). In 'Depressive illness: some research studies'. eds. Davies, B., Carroll, B.J. and Mowbray, R.M. C.C. Thomas, Springfield Illinois. pp 247-257.
- CARROLL, B.J., MOWBRAY, R.M. and DAVIES, B.M. (1970). Sequential comparison of L-tryptophan with ECT in severe depression. *Lancet*, i, 967-969.
- CARROLL, B.J., STEVEN, L., POPE, R.A. and DAVIES, B. (1969). Sodium transfer from plasma to CSF in severe depressive illness. *Arch. Gen. Psychiatry*, 21, 77-81.

- CAZZULLO, C.L., MANGONI, A. and MASCHERPA, G. (1966). Tryptophan metabolism in affective psychoses. *Br. J. Psychiatry*, 112, 157-162.
- CHAMBERS, D.A., SALZMAN, E.W. and NERI, L.L. (1967). Characterization of 'ecto-ATPase' of human blood platelets. *Arch. Biochem. Biophys.*, 119, 173-178.
- CHOI, S.J., TAYLOR, M.A. and ABRAMS, R. (1977). Depression, ECT and erythrocyte adenosinetriphosphatase activity. *Biol. Psychiatry*, 12, 75-81.
- CLARE, A. (1976). *Psychiatry in dissent. Controversial issues in thought and practice.* Tavistock Publications Ltd., London.
- COFFREY, R.G. and MIDDLETON, E., Jr. (1975). Increased adenosine triphosphatase activity in platelets of asthmatic children. *Int. Arch. Allergy Appl. Immunol.*, 48, 171-181.
- COHEN, N.S., EKHOLM, J.E., LUTHRA, M.G. and HANAHAN, D.G. (1975). Biochemical characterization of density-separated human erythrocytes. *Biochim. Biophys. Acta*, 419, 229-242.
- COHN, C.K., DUNNER, D.L. and AXELROD, J. (1970). Reduced catechol-O-methyltransferase activity in red blood cells of women with primary affective disorder. *Science*, 170, 1323-1324.
- COOPER, J.R., BLOOM, F.E. and ROTH, R.H. (1978). *The biochemical basis of neuropharmacology.* Oxford University Press, New York.
- COPPEN, A. (1960). Abnormality of the blood-cerebrospinal fluid barrier of patients suffering from a depressive illness. *J. Neurol. Neurosurg. Psychiatry*, 23, 156-161.
- COPPEN, A. (1967). Biochemistry of affective disorders. *Br. J. Psychiatry*, 113, 1237-1264.
- COPPEN, A. (1973). The role of lithium carbonate in the treatment and prophylaxis of the affective disorders. *Biochem. Soc. Trans.*, 1, 74-78.
- COPPEN, A. (1976). The effective dosage of antidepressants. *Postgrad. Med. J.*, 52 (Suppl.3), 72 - 74.
- COPPEN, A., BROOKSBANK, B.W.L. and ECCLESTON, E. (1974). Tryptophan metabolism in depressive illness. *Psychol. Med.*, 4, 164-173.
- COPPEN, A., BROOKSBANK, B.W.L. and PEET, M. (1972a). Tryptophan concentration in the cerebrospinal fluid of depressive patients. *Lancet*, i, 1393.
- COPPEN, A., ECCLESTON, E.G. and PEET, M. (1973). Total and free tryptophan concentration in the plasma of depressive patients. *Lancet*, ii, 60-63.
- COPPEN, A., GHOSE, K., SWADE, C. and WOOD, K. (1978). Effect of mianserin hydrochloride on peripheral uptake mechanisms for noradrenaline and 5-hydroxytryptamine in man. *Br. J. Clin. Pharmacol.* 5, 13S-17S.

- COPPEN, A., GUPTA, R., MONTGOMERY, S., GHOSE, K., BAILEY, J., BURNS, B. and DE RIDDER, J.J. (1976). Mianserin hydrochloride: a novel antidepressant. *Br. J. Psychiatry*, 129, 342-345.
- COPPEN, A., PRANGE, A.J., WHYBROW, P.C. and NOGUERA, R. (1972b). Abnormalities of indoleamines in affective disorders. *Arch. Gen. Psychiatry*, 26, 474-478.
- COPPEN, A.J. and SHAW, D.M. (1963). Mineral metabolism in melancholia. *Br. Med. J.*, ii, 1439-1444.
- COPPEN, A., SHAW, D.M., HERZBERG, B. and MAGGS, R. (1967). Tryptophan in treatment of depression. *Lancet*, ii, 1178-1180.
- COPPEN, A., SHAW, D.M. and MALLESON, A. (1965a). Changes in 5-hydroxytryptophan metabolism in depression. *Br. J. Psychiatry*, 11, 105-107.
- COPPEN, A.J., SHAW, D.M., MALLESON, A. and COSTAIN, R. (1966). Mineral metabolism in mania. *Br. Med. J.*, i, 71-75.
- COPPEN, A., SHAW, D.M., MALLESON, A., ECCLESTON, E. and GRUNDY, G. (1965b). Tryptamine metabolism in depression. *Br. J. Psychiatry*, 111, 996-998.
- COPPEN, A.J., SHAW, D.M. and MANGONI, A. (1962). Total exchangeable sodium in depressive illness. *Br. Med. J.*, ii, 295-298.
- COSTA, J.L. and MURPHY, D.L. (1975). Platelet 5-HT uptake and release stopped rapidly by formaldehyde. *Nature*, 255, 407-408.
- COSTA, J.L., MURPHY, D.L. and REVEILLE, J. (1977). Evaluation of the uptake of various amines into storage vesicles of intact human platelets. *Br. J. Pharmacol.*, 61, 223-228.
- COX, J.R., PEARSON, R.E. and SPEIGHT, C.J. (1971). Changes in sodium, potassium and body fluid spaces in depression and dementia. *Gerontol. Clin.*, 13, 233-245.
- CRANE, G.E. (1956). The psychiatric side-effects of iproniazid. *Am. J. Psychiatry*, 112, 494-501.
- CRANE, G.E. (1957). Further studies on iproniazid phosphate, -isonicotinil-isopropylhydrazine phosphate marsilid. *J. Nerv. Ment. Dis.*, 124, 322-331.
- CRAWFORD, N. (1965). The lungs and serotonin metabolism: a study of the pulmonary arterio-venous levels of plasma free and platelet-bound serotonin in man. *Clin. Chim. Acta*, 12, 264-273.
- CRAWFORD, N. (1967). Serotonin absorption by normal and "carcinoid" platelets. *Clin. Chim. Acta*, 18, 297-307.
- CREWS, F.T. and SMITH, C.B. (1978). Presynaptic alpha-receptor subsensitivity after long term antidepressant treatment. *Science*, 202, 322-324.

- CROUT, J.R. and SJOERDSMA, A. (1959). Clinical and laboratory significance of serotonin and catecholamine in bananas. *N. Engl. J. Med.*, 261, 23-26.
- CURZON, G. (1969). Tryptophan pyrrolase - a biochemical factor in depressive illness? *Br. J. Psychiatry*, 115, 1367-1374.
- CURZON, G. (1972). Brain amine metabolism in some neurological and psychiatric conditions. In 'Biochemical aspects of nervous diseases' ed. Cumings, J.N. Plenum Press, London and New York. pp 184-210.
- CURZON, G. and GREEN, A.R. (1968). Effect of hydrocortisone on rat brain 5-hydroxytryptamine. *Life Sci.*, 7, 657-663.
- DAVIS, J.M. (1970). Theories of biological etiology of affective disorders. *Int. Rev. Neurobiol.*, 12, 145-175.
- DAY, H.J., SCRUTTON, M. and HOLMSEN, H. (1973). Metal ions in human platelets: evidence for populations having differing contents of calcium and magnesium. *Fed. Proc.*, 32, 843.
- DEMONTIGNY, C. and AGHAJANIAN, G.K. (1978). Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. *Science*, 202, 1303-1305.
- DENKER, S.J., MALM, U., ROOS, B.E. and WERDINIUS, B. (1966). Acid monoamine metabolites of cerebrospinal fluid in mental depression and mania. *J. Neurochem.*, 13, 1545-1548.
- DESPOPOULOS, A. and WEISSBACH, H. (1957). Renal metabolism of 5-hydroxyindoleacetic acid. *Am. J. Physiol.*, 189, 548-550.
- DICK, D.A.T., DICK, E.G. and NAYLOR, G.J. (1981). Plasma vanadium concentration in manic-depressive illness. *J. Physiol. (Lond.)*, 310, 24P.
- DICK, D.A.T., DICK, E.G. and TOSTESON, D.C. (1969). Inhibition of adenosine triphosphatase in sheep red cell membranes by oxidised glutathione. *J. Gen. Physiol.*, 54, 123-133.
- DIXON, M. and WEBB, E.C. (1965). 'Enzymes'. Longmans, Green and Co. Ltd., London. pp 150-151.
- DRICKAMER, L.K. (1975). The red cell membrane contains three different adenosine triphosphatases. *J. Biol. Chem.*, 250, 1952-1954.
- DUNHAM, E.T. and GLYNN, I.M. (1961). Adenosinetriphosphatase activity and the active movements of alkali metal ions. *J. Physiol. (Lond.)*, 156, 274-293.
- DUNNER, D.L., COHN, C.K., GERSHON, E.S. and GOODWIN, F.K. (1971). Differential catechol-O-methyltransferase activity in unipolar and bipolar affective illness. *Arch. Gen. Psychiatry*, 25, 348-353.
- DUNNER, D.L. and FIEVE, R.R. (1975). Affective disorder. Studies with amine precursors. *Am. J. Psychiatry*, 132, 180-183.



- DUNNER, D.L. and GOODWIN, F.K. (1972). Effect of L-tryptophan on brain serotonin metabolism in depressed patients. *Arch. Gen. Psychiatry*, 26, 364-366.
- EBERT, M.H. and KOPIN, I.J. (1975). Differential labelling of origins of urinary catecholamine metabolites by dopamine-C14. *Trans. Assoc. Am. Physicians*, 88, 256-264.
- ECCLESTON, D., LOOSE, R., PULLAR, J.A. and SUGDEN, R.F. (1970). Exercise and urinary excretion of cyclic AMP. *Lancet*, ii, 612-613.
- EDWARDS, D.J. and BURNS, M.O. (1974). Effects of tricyclic anti-depressants upon human platelet monoamineoxidase. *Life Sci.*, 15, 2045-2048.
- ELEFOTHERION, B.E. and BOEHLKE, K.W. (1967). Brain monoamine oxidase in mice after exposure to aggression and defeat. *Science*, 155, 1693-1694.
- ERDMANN, E. and HASSE, W. (1975). Quantitative aspects of ouabain binding to human erythrocyte and cardiac membranes. *J. Physiol. (Lond.)*, 251, 671-682.
- ERWIN, G.V. (1973). Oxidative-reductive pathways for metabolism of biogenic aldehydes. In 'Frontiers of Catecholamine Research'. ed. Usdin, E. Pergamon Press, New York. pp 161-166.
- FEIG, S.A. and GUIDOTTI, G. (1974). Relative deficiency of  $\text{Ca}^{2+}$ -dependent adenosine triphosphatase activity of red cell membranes in hereditary spherocytosis. *Biochem. Biophys. Res. Commun.*, 58, 487-494.
- FEIGHNER, J.P., ROBINS, E., GUZE, S.B., WOODRUFF, R.A., WINOKUR, G. and MUNOZ, R. (1972). Diagnostic criteria for use in psychiatric research. *Arch. Gen. Psychiatry*, 26, 57-63.
- FERNSTROM, J.D. and WURTMAN, R.J. (1971). Brain serotonin content: Physiological dependence on plasma tryptophan levels. *Science*, 173, 149-152.
- FISCHER, E., HELLER, B. and MIRO, A.N. (1968).  $\beta$ -Phenylethylamine in human urine. *Arzneimittel. Forsch.*, 18, 1486.
- FISCHER, E., SPATZ, H., HELLER, B. and REGGIANI, H. (1972a). Phenylethylamine content of human urine and rat brain: Its alteration in pathological conditions and after drug administration. *Experientia*, 28, 307-308.
- FISCHER, E., SPATZ, H., SAAVERDRA, J.M., REGGIANI, H., MOIR, A.H. and HELLER, B. (1972b). Urinary excretion of phenylethylamine. *Biol. Psychiatry*, 5, 139-147.
- FOTHERBY, K., ASHCROFT, G.W., AFFLECK, J.W. and FORREST, A.D. (1963). Studies on sodium transfer and 5-hydroxyindoles in depressive illness. *J. Neurol. Neurosurg. Psychiatry*, 26, 71-73.

- FRAZER, A., MENDELS, J., BRUNSWICK, D. and RAMSAY, T.A. (1978). Transfer of sodium ions across the erythrocyte membrane in manic-depressive illness: treatment with lithium carbonate. *Life Sci.*, 22, 157-164.
- FRENCH, P.C., HOLMSEN, H. and STORMORKEN, H. (1970). Adenine nucleotide metabolism of blood platelets. VII. ATPases: sub-cellular localization and behaviour during the thrombin-platelet interaction. *Biochim. Biophys. Acta*, 206, 438-448.
- FRIZEL, D., COPPEN, A. and MARKS, V. (1969). Plasma magnesium and calcium in depression. *Br. J. Psychiatry*, 115, 1375-1377.
- FUKS, Z., LANMAN, R.C. and SCHANKER, L.S. (1964). On the membrane effects of chlorpromazine: uptake of biologic amines by the blood platelet and red cell. *Int. J. Neuropharmacol.*, 3, 623-633.
- GIBBONS, J.L. (1960). Total body sodium and potassium in depressive illness. *Clin. Sci.*, 19, 133-138.
- GILMAN, A.G., GOODMAN, L.S. and GILMAN, A. (1980). Goodman and Gilman's The Pharmacological Basis of Therapeutics. Sixth edition. MacMillan Publishing Co., Inc., New York.
- GLEN, A.I.M. and BELLINGER, L. (1973). Erythrocyte sodium and potassium ions in mania and depression. *Biochem. Soc. Trans.*, 1, 114-115.
- GLEN, A.I.M., ONGLEY, G.C. and ROBINSON, K. (1968). Diminished membrane transport in manic-depressive psychosis and recurrent depression. *Lancet*, ii, 241-243.
- GOODALL, M. and ALTON, H. (1968). Metabolism of 3-hydroxytryptamine (dopamine) in human subjects. *Biochem. Pharmacol.*, 17, 905-914.
- GOODALL, M. and ROSEN, L. (1963). Urinary excretion of noradrenaline and its metabolites at ten-minute intervals after intravenous injection of dl-noradrenaline-2-C. *J. Clin. Invest.*, 42, 1578-1588.
- GOODLET, I., MIREYLEES, S.E. and SUGRUE, M.F. (1977). Effects of mianserin, a new antidepressant, on the *in vitro* and *in vivo* uptake of monoamines. *Br. J. Pharmacol.*, 61, 307-313.
- GOODWIN, F.K. and BECKMANN, H. (1975). Urinary MHPG in unipolar and bipolar affective disorders. *Sci. Proc. Am. Psychiatr. Ass.*, 128, 96-97.
- GOODWIN, F.K., MURPHY, D.L., DUNNER, D.L. and BUNNEY, W.E., Jr. (1972). Lithium response in unipolar versus bipolar depression. *Am. J. Psychiatry*, 129, 44-47.
- GOODWIN, F.K. and POST, R.M. (1973). The use of probenecid in high doses for the estimation of central serotonin turnover in affective illness and addicts of methadone. In "Serotonin and Behaviour". eds. Barchas, J. and Usdin, E. Academic Press, New York. pp 469-480.
- GORDON, E.K., OLIVER, J., GOODWIN, F.K., CHASE, T.N. and POST, R.M. (1973). Effect of probenecid on free 3-methoxy-4-hydroxyphenyl-ethylene glycol (MHPG) and its sulphate in human cerebrospinal fluid. *Neuropharmacology*, 12, 391-396.

- GORDON, J.L. and OLVERMAN, H.J. (1977). Transport of dopamine by rat blood platelets. *Br. J. Pharmacol.*, 61, 142P-143P.
- GORDON, J.L. and OLVERMAN, H.J. (1978). 5-hydroxytryptamine and dopamine transport by rat and human blood platelets. *Br. J. Pharmacol.*, 62, 219-226.
- GOTTFRIES, C.G., ORELAND, L. and WIBERG, A. (1974). Brain-levels of monoamine oxidase in depression. *Lancet*, ii, 360.
- GOTTFRIES, C.G., ORELAND, L., WIBERG, A. and WIMBLAD, B. (1975). Lowered monoamine oxidase activity in brains from alcoholic suicides. *J. Neurochem.*, 25, 667-673.
- GRAHAME-SMITH, D.G., GREEN, A.R. and COSTAIN, D.W. (1978). Mechanism of the antidepressant action of electroconvulsive therapy. *Lancet*, i, 254-257.
- GREEN, A.R. and CURZON, G. (1968). Decrease of 5-hydroxytryptamine in the brain provoked by hydrocortisone and its prevention by allopurinol. *Nature*, 220, 1095-1097.
- GREEN, A.R. and GRAHAME-SMITH, D.G. (1975). 5-Hydroxytryptamine and other indoles in the central nervous system. In 'Handbook of Psychopharmacology'. Vol. 3. eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H. Plenum Press, New York. pp 169-245.
- GREENSPAN, K., SCHILDKRAUT, J.J., GORDON, E.K., BAER, L., ARANOFF, M.S. and DURELL, J. (1970). Catecholamine metabolism in affective disorders. III. MHPG and other catecholamine metabolites in patients treated with lithium carbonate. *J. Psychiatr. Res.*, 7, 171-183.
- GREENSPAN, K., SCHILDKRAUT, J.J., GORDON, E.K., LEVY, B. and DURELL, J. (1969). Catecholamine metabolism in affective disorders. II. Norepinephrine, normetanephrine, epinephrine, metanephrine and VMA excretion in hypomanic patients. *Arch. Gen. Psychiatry*, 21, 710-716.
- GROTE, S.S., MOSES, S.G., ROBINS, E. HUDGENS, R.W. and CRONINGER, A.B. (1974). A study of selected catecholamine-metabolizing enzymes: A comparison of depressive suicides and alcoholic suicides with controls. *J. Neurochem.*, 23, 791-802.
- GRUEN, P.H., SACHAR, E.J., ALTMAN, N. and SASSIN, J. (1975). Growth hormone responses to hypoglycemia in postmenopausal depressed women. *Arch. Gen. Psychiatry*, 32, 31-33.
- GULDBERG, H.C., ASHCROFT, G.W. and CRAWFORD, T.B.B. (1966). Concentrations of 5-hydroxyindoleacetic acid and homovanillic acid in the cerebrospinal fluid of the dog before and during treatment with probenecid. *Life Sci.*, 5, 1571-1575.
- GUPTA, J.D. and CROLLINI, C. (1975). Effect of lithium on magnesium-dependent enzymes. *Lancet*, i, 216-217.



- HALLSTROM, C.O.S., LINFORD REES, W., PARE, C.M.B., TRENCHARD, A. and TURNER, P. (1976). Platelet uptake of 5-hydroxytryptamine and dopamine in depression. *Postgrad. Med. J.*, 52 (Suppl.3), 40-44.
- HAMILTON, M. (1960). A rating scale for depression. *J. Neurol., Neurosurg. and Psychiatry*, 23, 56-62.
- HANAHAN, D.J. (1973). The erythrocyte membrane variability and membrane enzyme activity. *Biochim. Biophys. Acta*, 300, 319-340.
- HANAHAN, D.J. and EKHOLM, J. (1972). Changes in erythrocyte membranes during preparation, as expressed by ATPase activity. *Biochim. Biophys. Acta*, 255, 413-419.
- HANAHAN, D.J., EKHOLM, J. and HILDENBRANDT, G. (1973). Biochemical variability of human erythrocyte membrane preparations, as demonstrated by sodium-potassium-magnesium and calcium adenosine triphosphatase activities. *Biochemistry*, 12, 1375-1387.
- HANSON, J.P., REPKE, D.I., KATZ, A.M. and ALEDORT, L.M. (1973). Calcium ion control of platelet thrombosthenin ATPase activity. *Biochim. Biophys. Acta*, 314, 382-389.
- HARRIS, J.W. and KELLERMEYER, R.W. (1970). The red blood cell; production, metabolism and destruction; normal and abnormal. Harvard University Press.
- HESKETH, J.E. (1976). Changes in membrane adenosine triphosphatases on administration of lithium salts *in vivo*. *Biochem. Soc. Trans.*, 4, 328-330.
- HESKETH, J.E., GLEN, A.I.M. and READING, H.W. (1977). Membrane ATPase activities in depressive illness. *J. Neurochem.*, 28, 1401-1402.
- HESKETH, J.E., LOUDON, J.B., READING, H.W. and GLEN, A.I.M. (1978). The effect of lithium treatment on erythrocyte membrane ATPase activities and erythrocyte ion content. *Br. J. Clin. Pharmacol.*, 5, 323-329.
- HESKETH, J.E. and READING, H.W. (1978). Variation in adenosine triphosphatase activities in serial preparations of human erythrocyte membranes. *Biochem. Soc. Trans.*, 6, 918-920.
- HOKIN-NEAVERSON, M., SPIEGEL, D.A. and LEWIS, W.C. (1974). Deficiency of erythrocyte sodium pump activity in manic-depressive psychosis. *Life Sci.*, 15, 1739-1748.
- HOLMSEN, H., DAY, H.J. and STORMORKEN, H. (1969). The blood platelet release reaction. *Scand. J. Haematol.* (Suppl.) 8.
- HUGHES, F.B. and BRODIE, B.B. (1959). The mechanism of serotonin and catecholamine uptake by platelets. *J. Pharmacol. Exp. Ther.*, 127, 96-103.
- HULLIN, R.P. (1975). The effects of lithium on electrolyte balance and body fluids. In 'Lithium research and therapy'. ed. Johnson, F.N. Acad. Press, London. pp 359-379.

- HULLIN, R.P. (1976). Metabolism of indole amines in depression. *Postgrad. Med. J.*, 52 (Suppl. 3), 18-23.
- HULLIN, R.P., BAILEY, A.D., McDONALD, R., DRANSFIELD, G.A. and MILNE, H.B. (1967). Variations in 11-hydroxycorticosteroids in depression and manic-depressive psychosis. *Br. J. Psychiatry*, 113, 593-600.
- IMANDT, L., GENDERS, T., WESSELS, H. and HAANEN, C. (1977). The effect of lithium on platelet aggregation and platelet release reaction. *Thromb. Res.*, 11, 297-308.
- IMPASTATO, D. (1960). The story of the first electroshock treatment. *Am. J. Psychiatry*, 116, 1113-1114.
- ITIL, T.M. (1975). The GB94 story. In 'Proceedings of a symposium on depressive illness and experiences with a new antidepressant drug, GB94'. ed. Vossenaar, T. Excerpta Medica, Amsterdam. pp 9-21.
- ITIL, T.M., POLVAN, N. and HSU, W. (1972). Clinical and EEG effects of GB94, a tetracyclic antidepressant. *Curr. Therap. Res.*, 14, 395-413.
- JAIN, M. (1976). Neuropsychobiology of affective disorders. *Neuropsychobiology*, 2, 247-257.
- JANOWSKI, D.S., EL-YOUSEF, M.K. and DAVIS, J.M. (1974). Acetylcholine and depression. *Psychosom. Med.*, 36, 248-257.
- JANOWSKI, D.S., EL-YOUSEF, M.K., DAVIS, J.M. and SEKERKE, H.J. (1972). A cholinergic adrenergic hypothesis of mania and depression. *Lancet*, ii, 632-635.
- JANOWSKI, D.S., EL-YOUSEF, M.K., DAVIS, J.M. and SEKERKE, H.J. (1973). Parasympathetic suppression of manic syndromes by physostigmine. *Arch. Gen. Psychiatry*, 28, 542-552.
- JARRETT, H.W. and PENNISTON, J.T. (1978). Purification of the  $\text{Ca}^{2+}$ -stimulated ATPase activator from human erythrocytes. Its membership in the class of  $\text{Ca}^{2+}$ -binding modulator proteins. *J. Biol. Chem.*, 253, 4676-4682.
- JASKARI, M.O., AHLFORS, U.G., GINMAN, L., LYDECKEN, K. and TIENARI, P. (1977). Three double-blind comparative trials of mianserin (Org GB94) and amitriptyline in the treatment of depressive illness. *Pharmakopsychiatr. Neuropsychopharmakol.*, 10, 101-103.
- JENNER, F.A., GJESSING, L.R., COX, J.R., DAVIES-JONES, A., HULLIN, R.P. and HANNA, S.M. (1967). A manic depressive psychotic with a persistent forty-eight hour cycle. *Br. J. Psychiatry*, 113, 895-910.
- JENSEN, K., FRUENSGAARD, K., AHLFORS, U.G., PIHKANEN, T.A., TUOMIKOSKI, S., OSE, E., DENCKER, S.J., LINDBERG, D. and NAGY, A. (1975). Tryptophan/imipramine in depression. *Lancet*, ii, 920.

- JEPSON, J.B., LOVENBERG, W. and ZALTZMAN, P. (1960). Amine metabolism studied in normal and phenylketonuric humans by monoamine oxidase inhibition. *Biochem. J.*, 74, 5.
- JOHNSON, F.N. and CADE, J.F.J. (1975). Historical background to lithium research and therapy. In 'Lithium research and therapy'. ed. Johnson, F.N. Acad. Press, London. pp 9-22.
- JONES, F.D., MAAS, J.W., DEKIRMENJIAN, H. and FAWCETT, J.A. (1973). Urinary catecholamine metabolites during behavioural changes in a patient with manic-depressive cycles. *Science*, 179, 300-302.
- JONES, P.C.T. (1966). A contractile protein model for cell adhesion. *Nature*, 212, 365-369.
- JORI, A., DOLFINI, E., CASATI, C. and ARGENTA, G. (1975). Effect of ECT and imipramine treatment on the concentration of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) in the cerebrospinal fluid of depressed patients. *Psychopharmacologia (Berl.)*, 44, 87-90.
- JOSEPH, M.H. (1978). Brain tryptophan metabolism on the 5-hydroxytryptamine and kynurenine pathways in a strain of rats with a deficiency in platelet 5-HT. *Br. J. Pharmacol.*, 63, 529-533.
- KAFOE, W.F., DE RIDDER, J.J. and LEONARD, B.E. (1976). The effect of a tetracyclic antidepressant compound, Org GB94, on the turnover of biogenic amines in rat brain. *Biochem. Pharmacol.*, 25, 2455-2460.
- KANSAL, P.C., BUSE, J., TALBERT, O.R. and BUSE, M.G. (1972). Effect of L-dopa on plasma growth hormone, insulin, and thyroxine. *J. Clin. Endocrinol. Metab.*, 34, 99-105.
- KARKI, N.T. (1956). Urinary excretion of noradrenaline and adrenaline in different age groups. Its diurnal variation and effect of muscular work on it. *Acta Physiol. Scand.*, 39 (Suppl. 132), 5-96.
- KARPATKIN, S. and AMOROSI, E.L. (1977). Platelet heterogeneity. *Br. J. Haematology*, 35, 681-682.
- KATZ, B. and MILEDI, R. (1970). Further study of the role of calcium in synaptic transmission. *J. Physiol. (Lond.)*, 207, 789-801.
- KENDELL, R.E. (1978). Classification of depressive illness. *Scott. Med. J.*, 23, 61-63.
- KETY, S.S. (1971). Brain amines and affective disorders. In 'Brain Biochemistry and Mental Disease.' eds. Ho, B.T. and Mclsaac, W.M. Plenum Press, New York. pp 237-263.
- KIELHOLZ, P. and BATTEGAY, R. (1958). Behandlung depressiver Zustandsbilder, Unter spezieller Berücksichtigung von Tofranil, einem neuen Antidepressivum. *Schweiz. Med. Wochenschr.*, 88, 763-767.

- KINLOUGH-RATHBONE, R.L., MUSTARD, J.F., PACKHAM, M.A., PERRY, D.W., REIMERS, H.J. and CAZENAVE, J.P. (1977). Properties of washed human platelets. *Thromb. Haemost.*, 37, 291-308.
- KLEIN, R. (1950). Clinical and biochemical investigations in manic-depressive with short cycles. *J. Ment. Sc.*, 96, 293-297.
- KLEIN, R. and NUNN, R.F. (1945). Clinical and biochemical analysis of case of manic-depressive psychosis showing regular weekly cycles. *J. Ment. Sc.*, 91, 79-88.
- KLEINBERG, D.L., NOEL, G.L. and FRANTZ, A.G. (1971). Chlorpromazine stimulation and L-dopa suppression of plasma prolactin in man. *J. Clin. Endocrinol. Metab.*, 33, 873-876.
- KNOX, W.E. and AUERBACH, V.H. (1955). The hormonal control of tryptophan peroxidase in the rat. *J. Biol. Chem.*, 214, 307-313.
- KORF, J., VAN PRAAG, H.M. and SEBENS, J.B. (1971). Effect of intravenously administered probenecid in humans on the levels of 5-hydroxy-indole-acetic acid, homovanillic acid and 3-methoxy-4-hydroxy-phenyl-glycol in cerebrospinal fluid. *Biochem. Pharmacol.*, 20, 659-668.
- KORF, J., VAN PRAAG, H.M. and SEBENS, J.B. (1972). Serum tryptophan decreased, brain tryptophan increased and brain serotonin synthesis unchanged after probenecid loading. *Brain Res.*, 42, 239-242.
- KRISHNA, G., WEISS, B. and BRODIE, B.B. (1968). A simple sensitive method for the assay of adenylyl cyclase. *J. Pharmacol. Exp. Ther.*, 153, 379-385.
- KUHN, R. (1957). Über die Behandlung depressiver Zustände mit einem Iminodibenzylderivat (G22355). *Schweiz. Med. Wochenschr.*, 87, 1135-1140.
- KUHN, R. (1958). The treatment of depressive states with G22355 (imipramine hydrochloride). *Am. J. Psychiatry*, 115, 459-464.
- LAPIN, I.P. and OXENKRUG, G.F. (1969). Intensification of the central serotonergic process as a possible determinant of the thymoleptic effect. *Lancet*, i, 132-136.
- LE FUR, G. and UZAN, A. (1977). Effects of 4-(3-indolyl-alkyl) piperidine derivatives on uptake and release of noradrenaline, dopamine and 5-hydroxytryptamine in rat brain synaptosomes, rat heart and human blood platelets. *Biochem. Pharmacol.*, 26, 497-503.
- LEONARD, B.E. (1974). Some effects of a new tetracyclic antidepressant compound, Org GB94, on the metabolism of monoamines in the rat brain. *Psychopharmacologia (Berl.)*, 36, 221-236.
- LEONARD, B.E. and KAFOE, W.F. (1976). A comparison of the acute and chronic effects of four antidepressant drugs on the turnover of serotonin, dopamine and noradrenaline in the rat brain. *Biochem. Pharmacol.*, 25, 1939-1942.

- LEWANDER, T. and SJÖSTRÖM, R. (1973). Increase in the plasma concentration of free tryptophan caused by probenecid in humans. *Psychopharmacologia*, 33, 81-86.
- LEWIS, A. (1938). States of depression: their clinical and aetiological differentiation. *Br. Med. J.*, ii, 875-878.
- LINEWEAVER, H. and BURKE, H. (1934). The determination of enzyme dissociation constants. *J. Am. Chem. Soc.*, 56, 658-666.
- LINGJAERDE, O., Jr. (1969). Uptake of serotonin in blood platelets: dependence on sodium and chloride, and inhibition by choline. *FEBS Lett.*, 3, 103-106.
- LINGJAERDE, O., Jr. (1971). Studies on the uptake of serotonin in human blood platelets *in vitro*. Universitetsforlaget Trykningssentral, Oslo.
- LIPSETT, D., MADRAS, B.K., WURTMAN, R.J. and MUNRO, H.N. (1973). Serum tryptophan level after carbohydrate ingestion: Selective decline in non-albumin-bound tryptophan coincident with reduction in serum free fatty acids. *Life Sci.*, 12, 57-64.
- LLOYD, K.G., FARLEY, I.J., DECK, J.H.N. and HORNYKIEWICZ, O. (1974). Serotonin and 5-hydroxyindoleacetic acid in discrete areas of the brainstem of suicide victims and control patients. *Adv. Biochem. Psychopharmacol.*, 11, 387-397.
- LOOMER, H.P., SAUNDERS, J.C. and KLINE, N.S. (1957). A clinical and pharmaco-dynamic evaluation of iproniazid as a psychic energizer. *Psychiatr. Res. Rep.*, 8, 129-141.
- LOVENBERG, W. and ENGELMAN, K. (1971). Assay of serotonin, related metabolites and enzymes. *Methods Biochem. Anal.*, 19, 1-34.
- LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275.
- MAAS, J.W., DEKIRMENJIAN, H. and FAWCETT, J. (1971). Catecholamine metabolism, depression and stress. *Nature*, 230, 330-331.
- MAAS, J.W., DEKIRMENJIAN, H., GARVER, D., REDMON, D.E., Jr., and LANDIS, D.H. (1973a). Excretion of catecholamine metabolites following intraventricular injection of 6-hydroxydopamine in the *Macaca speciosa*. *Eur. J. Pharmacol.*, 23, 121-130.
- MAAS, J.W.H., DEKIRMENJIAN, H. and JONES, F. (1973b). The identification of depressed patients who have a disorder of NE metabolism and/or disposition. In 'Frontiers in Catecholamine Research'. eds. Usdin, E. and Snyder, S. Pergamon Press, New York. pp 1091-1096.
- MAAS, J.W., FAWCETT, J.A. and DEKIRMENJIAN, H. (1968). 3-Methoxy-4-hydroxyphenylglycol (MHPG) excretion in depressive states: Pilot study. *Arch. Gen. Psychiatry*, 19, 129-134.



- MAAS, J.W. and LANDIS, D.H. (1968). *In vivo* studies of the metabolism of norepinephrine in the central nervous system. *J. Pharmacol. Exp. Ther.*, 163, 147-162.
- MAGGI, A. and ENNA, S.J. (1980). Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. *J. Neurochem.*, 34, 888-892.
- MARCUS, A.J. and ZUCKER, M.B. (1965). The physiology of blood platelets. Grune and Stratton, New York.
- MARSHALL, E.F., STIRLING, G.S., TAIT, A.C. and TODRICK, A. (1960). The effect of iproniazid and imipramine on the blood platelet 5-hydroxytryptamine level in man. *Br. J. Pharmacol.*, 15, 35-41.
- MARTIN, J.B. (1973). Neural regulations of growth hormone secretion. *N. Engl. J. Med.*, 288, 1384-1393.
- MASON, R.G. and SABA, S.R. (1969). Platelet ATPase activities. I. Ecto-ATPases of intact platelets and their possible role in aggregation. *Am. J. Pathol.*, 55, 215-223.
- McCOY, E.E., SEGAL, D.J., BAYER, S.M. and STRYNADKA, K.D. (1974). Decreased ATPase and increased sodium content of platelets in Down's syndrome. Relation to decreased serotonin content. *N. Engl. J. Med.*, 291, 950-953.
- McNAMEE, H.B., MOODY, J.P. and NAYLOR, G.J. (1972). Indoleamine metabolism in affective disorders: Excretion of tryptamine, indoleacetic acid and 5-hydroxyindoleacetic acid in depressive states. *J. Psychosom. Res.*, 16, 63-70.
- MEDICAL RESEARCH COUNCIL (1965). Report by clinical psychiatry committee. *Br. Med. J.*, i, 881-886.
- MEDUNA, L.V. (1938). General discussion of the cardiazol therapy. *Am. J. Psychiatry*, 94 (Suppl.), 40-50.
- MEITES, J., LU, K.H., WUTTKE, W., WELSCH, C.W., NAGASAWA, H. and QUADRIE, F.K. (1972). Recent studies on function and control of prolactin secretion in rats. *Recent Prog. Horm. Res.*, 28, 471-526.
- MENDELS, J. (1975). Lithium in the acute treatment of depressive states. In 'Lithium research and therapy'. ed. Johnson, F.N. Academic Press, London. pp 43-62.
- MENDELS, J. and FRAZER, A. (1974). Alterations in cell membrane activity in depression. *Am. J. Psychiatry*, 131, 1240-1246.
- MENDELS, J., FRAZER, A., FITZGERALD, R.G., RAMSAY, T.A. and STOKES, J.W. (1972a). Biogenic amine metabolites in cerebrospinal fluid of depressed and manic patients. *Science*, 175, 1380-1382.
- MENDELS, J., FRAZER, A. and SECUNDA, S.K. (1972b). Intra-erythrocyte sodium and potassium in manic-depressive illness. *Biol. Psychiatry*, 5, 165-171.

- MENDELS, J., FRAZER, A., SECUNDA, S.K. and STOKES, J.W. (1971). Biochemical changes in depression. *Lancet*, i, 448-449.
- MENDELS, J., SECUNDA, S.K. and DYSON, W. (1972c). A controlled study of the antidepressant effects of lithium carbonate. *Arch. Gen. Psychiatry*, 26, 154-157.
- MENDELS, J., STINNET, J.L., BURNS, D. and FRAZER, A. (1975). Amine precursors and depression. *Arch. Gen. Psychiatry*, 32, 22-30.
- MENDLEWICZ, J. and RAINER, J.D. (1977). Adoption study supporting genetic transmission in manic-depressive illness. *Nature*, 268, 327-329.
- MESSIHA, F.S., AGALLIANOS, D. and CLOWER, C. (1970). Dopamine excretion in affective states and following  $\text{Li}_2\text{CO}_3$  therapy. *Nature*, 225, 868-869.
- MILLER, E.E., SAWANO, S. and ARIMURA, A. (1967). Blockade of release of growth hormone by brain norepinephrine depletors. *Endocrinology*, 80, 471-476.
- MOAKE, J.L., AHMED, K., BACHUR, N.R. and GUTFREUND, D.E. (1970).  $\text{Mg}^{2+}$ -dependent,  $(\text{Na}^+ + \text{K}^+)$ -stimulated ATPase of human platelets. Properties and inhibition by ADP. *Biochim. Biophys. Acta*, 211, 337-344.
- MONTGOMERY, S., MCAULEY, R. and MONTGOMERY, D.B. (1978). Relationship between mainserin plasma levels and antidepressant effect in a double-blind trial comparing a single night-time and divided daily dose regimens. *Br. J. Clin. Pharmacol.*, 5, 71S-76S.
- MOSNAIM, A.D., INWANG, E.E. and SABELLI, H.C. (1974). The influence of psychotropic drugs on the levels of endogenous 2-phenylethylamine in the rabbit brain. *Biol. Psychiatry*, 8, 227-234.
- MOYES, I.C.A. and MOYES, R.B. (1976). Further developments in the study of 3',5' cyclic adenosine monophosphate in relation to psychiatric illness. *Postgrad. Med. J.*, 52 (Suppl. 3), 110-114.
- MULGIRIGAMA, L.D. (1976). Significance of uptake studies with biogenic amines. In 'Research and clinical investigation in depression'. ed. Murphy, J.E. Cambridge Med. Pub., Northampton, England. pp 10-15.
- MÜLLER, E.E., PECILE, A., FELICI, M. and COCCHI, D. (1970). Norepinephrine and dopamine injection into lateral brain ventricles of the rat and growth hormone releasing activity in hypothalamus and plasma. *Endocrinology*, 86, 1376-1382.
- MURPHY, D.L. (1972). Amine precursors, amines, and false neurotransmitters in depressed patients. *Am. J. Psychiatry*, 129, 141-148.
- MURPHY, D.L., COLBURN, R.W., DAVIS, J.M. and BUNNEY, W.E., Jr. (1969). Stimulation by lithium of monoamine uptake in human platelets. *Life Sci.*, 8, 1187-1193.

- MURPHY, D.L., COLBURN, R.W., DAVIS, J.M. and BUNNEY, W.E., Jr. (1970). Imipramine and lithium effects on biogenic amine transport in depressed and manic-depressed patients. *Am. J. Psychiatry*, 127, 339-344.
- MURPHY, D.L. and WEISS, R. (1972). Reduced monoamine oxidase activity in blood platelets from bipolar depressed patients. *Am. J. Psychiatry*, 128, 35-41.
- MURPHY, J.E. (1975). A comparative clinical trial of Org GB94 and imipramine in the treatment of depression in general practice. *J. Int. Med. Res.*, 3, 251-260.
- MURPHY, J.E., DONALD, J.F. and MOLLA, A.L. (1976). Mianserin in the treatment of depression in general practice. *Practitioner*, 217, 135-138.
- NAKAO, T., NAGANO, K., ADACHI, K. and NAKAO, M. (1963). Separation of two adenosine triphosphatases from erythrocyte membrane. *Biochem. Biophys. Res. Commun.*, 13, 444-448.
- NAYLOR, G.J., BUCKLEY, D.E., BOARDMAN, L.J., SMITH, A.H.W. and MOODY, J.P. (1978). Attempt to demonstrate an *in vivo* effect of mianserin hydrochloride on erythrocyte  $\text{Na}^+/\text{K}^+$ -ATPase activity and cyclic AMP concentration. *Br. J. Clin. Pharmacol.*, 5, 49S-51S.
- NAYLOR, G.J., DICK, D.A.T., DICK, E.G., LE POIDEVIN, D. and WHYTE, S.F. (1973). Erythrocyte membrane cation carrier in depressive illness. *Psychol. Med.*, 3, 502-508.
- NAYLOR, G.J., DICK, D.A.T., DICK, E.G. and MOODY, J.P. (1974). Lithium therapy and erythrocyte membrane cation carrier. *Psychopharmacologia*, 37, 81-86.
- NAYLOR, G.J., MCNAMEE, H.B. and MOODY, J.P. (1970a). Erythrocyte sodium and potassium in depressive illness. *J. Psychosom. Res.*, 14, 173-177.
- NAYLOR, G.T., MCNAMEE, H.B. and MOODY, J.P. (1970b). The plasma control of erythrocyte sodium and potassium metabolism in depressive illness. *J. Psychosom. Res.*, 14, 179-186.
- NAYLOR, G.T., MCNAMEE, H.B. and MOODY, J.P. (1971). Changes in erythrocyte sodium and potassium on recovery from a depressive illness. *Br. J. Psychiatry*, 118, 219-223.
- NAYLOR, G.J. and SMITH, A.H.W. (1981). Vanadium: a possible aetiological factor in manic depressive illness. *Psychol. Med.*, 11, 249-256.
- NAYLOR, G.J., SMITH, A.H.W., DICK, E.G., DICK, D.A.T., MCHARG, A.M. and CHAMBERS, C.A. (1980). Erythrocyte membrane cation carrier in manic-depressive psychosis. *Psychol. Med.*, 10, 521-525.



- NEFF, N.H., TOZER, T.H. and BRODIE, B.B. (1967). Application of steady-state kinetics to studies of the transfer of 5-hydroxy-indoleacetic acid from brain to plasma. *J. Pharmacol. Exp. Ther.*, 158, 214-218.
- NEFF, N.H. and YANG, H.Y.T. (1974). Another look at the monoamine oxidases and the monoamine oxidase inhibitor drugs. *Life Sci.*, 14, 2061-2077.
- NIES, A., ROBINSON, D.S. and HARRIS, L.S. (1974). Comparison of monoamine oxidase substrate activities in twins, schizophrenics, depressives, and controls. *Adv. Biochem. Psychopharmacol.*, 12, 59-70.
- NORDIN, G., OTTOSSON, J.O. and ROOS, B.E. (1971). Influence of convulsive therapy on 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid in endogenous depression. *Psychopharmacologia*, 20, 315-320.
- OVERALL, J.E., HOLLISTER, L.E., MEYER, F., KIMBALL, I. and SHELTON, J. (1964). Imipramine and thioridazine in depressed and schizophrenic patients. *J.A.M.A.*, 189, 93-96.
- PAPESCHI, R. and McCLURE, D.J. (1971). Homovanillic acid and 5-hydroxyindoleacetic acid in cerebrospinal fluid of depressed patients. *Arch. Gen. Psychiatry*, 25, 354-358.
- PARE, C.M.B. and SANDLER, M. (1959). A clinical and biochemical study of a trial of iproniazid in the treatment of depression. *J. Neurol. Neurosurg. Psychiatry*, 22, 247-251.
- PARE, C.M.B., YEUNG, D.P.H., PRICE, K. and STACEY, R.S. (1969). 5-Hydroxytryptamine in brainstem, hypothalamus and caudate nucleus of controls and of patients committing suicide by coal-gas poisoning. *Lancet*, ii, 133-135.
- PAUL, M.J., CRAMER, H. and GOODWIN, F.K. (1971). Urinary cyclic AMP excretion in depression and mania. *Arch. Gen. Psychiatry*, 24, 327-333.
- PAUL, M.J., DITZION, B.R., PAUK, G.L. and JANOWSKI, D.S. (1970). Urinary adenosine 3'5'-monophosphate excretion in affective disorders. *Am. J. Psychiatry*, 126, 1493-1497.
- PEET, M. (1975). Lithium in the acute treatment of mania. In 'Lithium research and therapy'. ed. Johnson, F.N. Academic Press, London. pp 25-41.
- PEET, M. and BEHAGEL, H. (1978). Mianserin: a decade of scientific development. *Br. J. Clin. Pharmacol.*, 5, 5S-9S.
- PERRY, G.F., FITZSIMMONS, B., SHAPIRO, L. and IRWIN, P. (1978). Clinical study of mianserin, imipramine and placebo in depression: blood level and MHPG correlations. *Br. J. Clin. Pharmacol.*, 5, 35S-41S.
- PICHOT, P., DREYFUS, J.F. and PULL, C. (1978). A double-blind multicentre trial comparing mianserin with imipramine. *Br. J. Clin. Pharmacol.*, 5, 87S-90S.

- PLETSCHER, A. (1968). Metabolism, transfer and storage of 5-HT in blood granules. *Br. J. Pharmacol.*, 32, 1-16.
- PLETSCHER, A., BARTHOLINI, G. and DA PRADA, M. (1966). Metabolism of monoamines by blood platelets and relation to 5-hydroxytryptamine liberation. In 'Mechanisms of release of biogenic amines'. Pergamon Press, London and New York.
- PLETSCHER, A., DA PRADA, M., BERNEIS, K.H. and TRANZER, J.P. (1971). New aspects on the storage of 5-hydroxytryptamine in blood platelets. *Experientia*, 27, 993-1002.
- PLOEGER, E.J. (1974). The effects of lithium on excitable cell membranes on the mechanism of inhibition of the sodium pump of non-myelinated nerve fibres of the rat. *Eur. J. Pharmacol.*, 25, 316-321.
- PORSOLT, R.D., LE PICHON, M. and JALFRE, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, 266, 730-732.
- POST, R.M., KOTIN, J., GOODWIN, F.K. and GORDON, E.K. (1973). Psychomotor activity and cerebrospinal fluid amine metabolites in affective illness. *Am. J. Psychiatry*, 130, 67-72.
- PRANGE, A.J., Jr., McCURDY, L.R. and COCHRANE, C.M. (1967). The systolic blood pressure response of depressed patients to infused norepinephrine. *J. Psychiatr. Res.*, 5, 1-13.
- PRANGE, A.J., Jr., WILSON, I.C., LYNN, C.W., ALLTOP, L.B., STIKELEATHER, R.A. and RALEIGH, N.C. (1974). L-Tryptophan in mania. *Arch. Gen. Psychiatry*, 30, 52-62.
- RAHAMIMOFF, R., RAHAMIMOFF, H., BINA, O. and MEIRI, U. (1975). Control of neurotransmitter release by calcium ions and the role of mitochondria. In 'Calcium transport in contraction and secretion'. eds. Carafoli, E., Clementi, F., Drabikowski, W. and Margreth, A. North Holland Publ. Co. Ltd., Oxford. pp 253-260.
- RAISON, J.K., LYONS, J.M., MEHLFORD, R.J. and KEITH, A.D. (1971). Temperature-induced phase changes in mitochondrial membranes detected by spin labelling. *J. Biol. Chem.*, 246, 4036-4040.
- RAITERI, M., ANGELINI, F. and BERTOLLINI, A. (1976). Comparative study of the effects of mianserin, a tetracyclic antidepressant, and of imipramine on uptake and release of neurotransmitters in synaptosomes. *J. Pharm. Pharmacol.*, 28, 483-488.
- REES, J.R., ALLTOP, M.N.E. and HULLIN, R.P. (1974). Plasma concentrations of tryptophan and other amino acids in manic-depressive patients. *Psychol. Med.*, 4, 334-337.
- RICHMOND, J. and DAVIES, S.H. (1968). Blood. In 'A companion to medical studies', Vol. 1. Blackwell Scientific Publ., Oxford and Edinburgh. pp 26.1-26.20.

- RIDGES, A.P. (1976). The potential value of biochemical parameters in the diagnosis and medication of affective disorders. *Postgrad. Med. J.*, 52 (Suppl. 3), 9-16.
- RINNE, U.K. and SONNINEN, V. (1968). A double blind study of L-dopa treatment in Parkinson's disease. *Europ. Neurol.*, 1, 180-191.
- ROBBLEE, L.S., SHEPRO, D. and BELAMARICH, F.A. (1973). Calcium uptake and associated adenosine triphosphatase activity of isolated platelet membranes. *J. Gen. Physiol.*, 61, 462-481.
- ROBINSON, C.W., Jr., KRESS, S.C., WAGNER, R.H. and BRINKHOUS, K.M. (1965). Platelet agglutination and deagglutination with a sulfhydryl inhibitor, methyl mercuric nitrate: relationships to platelet ATPase. *Exp. Mol. Pathol.*, 4, 457-464.
- ROBINSON, D.S., DAVIS, J.M., NIES, A., RAVARIS, C.G. and SYLWESTER, D. (1971). Relation of sex and aging to monoamine oxidase activity of human brain, plasma and platelets. *Arch. Gen. Psychiatry*, 24, 536-539.
- ROOS, B.E. and SJÖSTRÖM, R. (1969). 5-Hydroxyindoleacetic acid and homovanillic acid levels in the cerebrospinal fluid after probenecid application in patients with manic-depressive psychosis. *J. Clin. Pharmacol.*, 1, 153-155.
- ROTH, J.A. and GILLIS, C.N. (1974). Deamination of  $\beta$ -phenylethylamine by monoamine oxidase-inhibition by imipramine. *Biochem. Pharmacol.*, 23, 2537-2545.
- RUBIN, R.T. (1967). Adrenal cortical activity changes in manic-depressive illness. *Arch. Gen. Psychiatry*, 17, 671-679.
- RUBIN, R.T., MILLER, R.G., CLARK, B.R., POLAND, R.E. and ARTHUR, R.J. (1970). The stress of aircraft carrier landings. II. 3-Methoxy-4-hydroxyphenylglycol excretion in naval aviators. *Psychosom. Med.*, 32, 589-597.
- RUSSELL, G.F.M. (1960). Body weight and balance of water, sodium and potassium in depressed patients given electro-convulsive therapy. *Clin. Sci.*, 19, 327-336.
- SABA, S.R., RODMAN, N.F. and MASON, R.G. (1969). Platelet ATPase activities. II. ATPase activities of isolated platelet membrane fractions. *Am. J. Pathol.*, 55, 225-233.
- SABELLI, H.C. and MOSNAIM, A.D. (1974). Phenylethylamine hypothesis of affective behaviour. *Am. J. Psychiatry*, 131, 695-699.
- SACHAR, E.J., FRANTZ, A.G., ALTMAN, N. and SASSIN, J. (1973). Growth hormone and prolactin in unipolar and bipolar depressed patients: Response to hypoglycemia and L-dopa. *Am. J. Psychiatry*, 130, 1362-1367.
- SALZMAN, F.W., CHAMBERS, D.A. and NERI, L.L. (1966). Possible mechanism of aggregation of blood platelets by adenosine diphosphate. *Nature*, 210, 167-169.

- SAUERBIER, I. and VON MAYERSBACH, H. (1976). Circadian variation in 5-hydroxytryptamine levels in human blood. *Chronobiologia*, 3, 131-136.
- SCATCHARD, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, 51, 660-672.
- SCHANBERG, S.M., SCHILDKRAUT, J.J., BREESE, G.R. and KOPIN, I.J. (1968). Metabolism of normetanephrine H<sup>3</sup> in rat brain: Identification of conjugated 3-methoxy-4-hydroxy-phenylglycol as major metabolite. *Biochem. Pharmacol.*, 17, 247-254.
- SCHATZMANN, H.J. (1975). Active calcium transport across the plasma membrane of erythrocytes. In 'Calcium transport in contraction and secretion'. eds. Carafoli, E., Clementi, F., Drabikowski, W. and Margreth, A. North Holland Publ. Co. Ltd., Oxford. pp 45-49.
- SCHILDKRAUT, J.J. (1965). The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am. J. Psychiatry*, 122, 509-522.
- SCHILDKRAUT, J.J. (1973). Neuropharmacology of the affective disorders. *Annu. Rev. Pharmacol.*, 13, 427-454.
- SCHILDKRAUT, J.J. (1975). Depressions and biogenic amines. In 'American Handbook of Psychiatry', Vol. VI. ed. Hamburg, D. Basic Books, New York. pp 609-672.
- SCHLESSER, M.A., WINOKUR, G. and SHERMAN, B.M. (1979). Genetic subtypes of unipolar primary depressive illness distinguished by hypothalamic-pituitary-adrenal axis activity. *Lancet*, i, 739-741.
- SCHOTTSTAEDT, W.W., GRACE, W.J. and WOLFF, H.G. (1956). Life situations, behaviour, attitudes, emotions and renal excretion of fluid and electrolytes. *J. Psychosom. Res.*, 1, 287-291.
- SCHOU, M. (1957). Biology and pharmacology of the lithium ion. *Pharmacol. Rev.*, 9, 17-58.
- SCHOU, M. (1973). Possible mechanisms of action of lithium salts: approaches and perspectives. *Biochem. Soc. Trans.*, 1, 81-87.
- SCHOU, M. and THOMSEN, K. (1975). Lithium prophylaxis of recurrent endogenous affective disorders. In 'Lithium research and therapy'. ed. Johnson, F.N. Acad. Press, London. pp 63-84.
- SCHWARTZ, A., LINDENMAYER, G.E. and ALLEN, J.C. (1975). The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacol. Rev.*, 27, 3-134.
- SHAW, D.M., CAMPS, F.E. and ECCLESTON, E.G. (1967). 5-Hydroxytryptamine in the hind-brain of depressive suicides. *Br. J. Psychiatry*, 113, 1407-1411.
- SHAW, D.M., FRIZEL, D., CAMPS, F.E. and WHITE, S. (1969). Brain electrolytes in depressive and alcoholic suicides. *Br. J. Psychiatry*, 115, 69-79.

- SIEGAL, A., BURRI, P.H., WEIBEL, E.R., BETTEX-GALLAND, M. and LÜSCHER, E.F. (1971). Density gradient centrifugation and electron microscopic characterization of subcellular fractions from human blood platelets. *Thromb. Diath. Haemorrh. (Stuttg.)*, 25, 252-267.
- SINANAN, K., KEATINE, A.M.B., BECKETT, P.G.S. and LOVE, W.C. (1975). Urinary cyclic AMP in "endogenous" and "neurotic" depression. *Br. J. Psychiatry*, 126, 49-55.
- SJÖSTRÖM, R. and ROOS, B.E. (1972). 5-Hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid in manic-depressive psychosis. *Eur. J. Clin. Pharmacol.*, 4, 170-176.
- SKOU, J.C. (1965). Enzymatic basis for active transport of  $\text{Na}^+$  and  $\text{K}^+$  across cell membranes. *Physiol. Rev.*, 45, 596-617.
- SLOANE, R.B., HUGHES, W. and HAUST, H.L. (1966). Catecholamine excretion in manic-depressive and schizophrenic psychosis and its relationship in symptomatology. *Can. Psychiatr. Assoc. J.*, 11, 6-19.
- SMITH, A.H.W., NAYLOR, G.S. and MOODY, J.P. (1978a). Placebo-controlled double-blind trial of mianserin hydrochloride. *Br. J. Clin. Pharmacol.*, 5, 67S-70S.
- SMITH, L.T., HANSON, D.R. and OMENN, G.S. (1978b). Comparisons of serotonin uptake by blood platelets and brain synaptosomes. *Brain Res.*, 146, 400-403.
- SNEDDON, J.M. (1969). Sodium-dependent accumulation of 5-hydroxytryptamine by rat blood platelets. *Br. J. Pharmacol.*, 37, 680-688.
- SNEDDON, J.M. (1971). Relationship between internal  $\text{Na}^+/\text{K}^+$  and the accumulation of  $^{14}\text{C}$ -5-hydroxytryptamine by rat platelets. *Br. J. Pharmacol.*, 43, 834-844.
- SNEDDON, J.M. (1973). Blood platelets as a model for monoamine-containing neurones. *Prog. Neurobiol.*, 1, 151-198.
- SOLOMON, H.M., SPIRT, N.M. and ABRAMS, W.B. (1970). The accumulation and metabolism of dopamine by the human platelet. *Clin. Pharmacol. Ther.*, 11, 838-845.
- STACEY, R.S. (1961). Uptake of 5-hydroxytryptamine by platelets. *Br. J. Pharmacol.*, 16, 284-295.
- STAHL, S.M. (1977). The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch. Gen. Psychiatry*, 34, 509-516.
- STAHL, S.M. and MELTZER, H.Y. (1978). A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: Comparison with central serotonergic neurons. *J. Pharmacol. Exp. Ther.*, 205, 118-132.
- STEIN, W.D. (1967). The movement of molecules across cell membranes. *Theoretical and Experimental Biology*, 6. Academic Press, London.
- STRÖM-OLSEN, R. and WEIL-MALHERBE, H. (1958). Humoral changes in manic-depressive psychosis with particular reference to excretion of catecholamines in urine. *J. Ment. Sci.*, 104, 696-704.



- SULSER, F. and ROBINSON, S.E., (1978). Clinical implications of pharmacological differences among antipsychotic drugs. In 'Psychopharmacology: A Generation of Progress'. eds. Lipton, M.A., Di Mascio, A. and Killam, K.F. Raven Press, New York. pp 943-954.
- SVENSSSEN, T.H. and USDIN, T. (1978). Feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants:  $\alpha$  receptor mediation. *Science*, 202, 1089-1091.
- TAKAHASHI, R., NAGAO, Y., TSUCHIYA, K., TAKAMIZAWA, M. and KOBAYASHI, T. (1968a). Catecholamine metabolism of manic-depressive illness. *J. Psychiatr. Res.*, 6, 185-199.
- TAKAHASHI, R., UTENA, H., MACHIYAMA, Y., KURIHAMA, M., OTSUKA, T., NAKAMURA, T. and KONAMURA, H. (1968b). Tyrosine metabolism in manic-depressive illness. *Life Sci.*, 7, 1219-1231.
- TANIGUCHI, K. and IIDA, S. (1972). The effect of phospholipids on the apparent activation energy of  $(\text{Na}^+ - \text{K}^+)\text{-ATPase}$ . *Biochim. Biophys. Acta*, 274, 536-541.
- TISSOT, R. (1962). Connaissances experimentales sur les monoamines et quelques syndromes psychiatriques. In 'Monoamines et Système Nerveux Central'. ed. de Ajuriaguerra, J. Georg and Cie, Geneva. pp 169-207.
- TODRICK, A. and TAIT, A.C. (1969). The inhibition of human platelet 5-hydroxytryptamine uptake by tricyclic antidepressive drugs. The relation between structure and potency. *J. Pharm. Pharmacol.*, 21, 751-762.
- TOIVOLA, P.T.K. and GALE, C.C. (1972). Stimulation of growth hormone release by microinjection of norepinephrine into hypothalamus of baboons. *Endocrinology*, 90, 895-902.
- TREISER, S. and KELLAR, K.J. (1979). Lithium effects on adrenergic receptor supersensitivity in rat brain. *Eur. J. Pharmacol.*, 58, 85-86.
- TUOMISTO, J. (1974). A new modification for studying 5-HT uptake by blood platelets: a re-evaluation of tricyclic antidepressants as uptake inhibitors. *J. Pharm. Pharmacol.*, 26, 92-100.
- TUOMISTO, J. and TUKIANEN, E. (1976). Decreased uptake of 5-hydroxytryptamine in blood platelets from depressed patients. *Nature*, 262, 596-598.
- TYCE, G.M., MUENTER, M.D. and OWEN, C.A. (1970). Metabolism of L-dihydroxyphenylalanine by patients with Parkinson's disease. *Mayo Clin. Proc.*, 45, 645-656.
- U'PRITCHARD, D.C., GREENBERG, D.A., SHEEHAN, P.P. and SNYDER, S.H. (1978). Tricyclic antidepressants: therapeutic properties and affinity for alpha-noradrenergic receptor binding sites in the brain. *Science*, 199, 197-198.
- VAN DER BURG, W.J., BONTA, I.L., DELOBELLE, J., RAMON, C. and VARGAFTIG, B. (1970). A novel type of substituted piperazine with high anti-serotonin potency. *J. Med. Chem.*, 1, 35-39.

- VAN PRAGG, H.M. (1977). Significance of biochemical parameters in the diagnosis, treatment and prevention of depressive disorders. *Biol. Psychiatry*, 12, 101-131.
- VAN PRAAG, H.M. (1978). Amine hypotheses of affective disorders. In 'Handbook of psychopharmacology'. Vol. 13. eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H. Plenum Press, New York. pp 187-297.
- VAN PRAAG, H.M., FLENTGE, F., KORF, J., DOLS, L.C.W. and SCHUT, T. (1973a). The influence of probenecid in the metabolism of serotonin, dopamine and their precursors in man. *Psychopharmacologia*, 33, 141-151.
- VAN PRAAG, H.M., KORF, J. and PUITE, J. (1970). 5-Hydroxyindoleacetic acid levels in the cerebrospinal fluid of depressive patients treated with probenecid. *Nature*, 225, 1259-1260.
- VAN PRAAG, H.M., KORF, J. and SCHUT, T. (1973b). Cerebral monoamines and depression. An investigation with the probenecid technique. *Arch. Gen. Psychiatry*, 28, 827-831.
- VAN PRAAG, H.M. and LEIJNSE, B. (1963a). Die Bedeutung der Monoaminoxidasehemmung als antidepressives Prinzip I. *Psychopharmacologia*, 4, 1-14.
- VAN PRAAG, H.M. and LEIJNSE, B. (1963b). Die Bedeutung der Psychopharmacologie für die Klinische Psychiatrie. Systematik als notwendiger Ausgangspunkt. *Nervenarzt*, 34, 530-537.
- VOGEL, H.P., BENTE, D., FEDER, J., HELMCHEN, H., MÜLLER-OBERLINGHAUSEN, B., BOHACEK, N., MIHOVILOVIC, M., BRÄNLI, A., FLEISCHHAUSER, J.J. and WALCHER, W. (1976). Mianserin versus amitriptyline. A double-blind trial evaluated by the AMP system. *Int. Pharmacopsychiatry*, 11, 25-31.
- WALTON, K.G. (1977). Cyclic nucleotides and postsynaptic events. In 'Essays in Neurochemistry and Neuropharmacology', Vol. 2. eds. Youdim, M.B.H., Lovenberg, W., Sharman, D.F. and Lagnado, J.R. A Wiley-Interscience Publication. Chap. 5, pp 105-149.
- WANG, Y.C., PANDEY, G.N., MENDELS, J. and FRAZER, A. (1974a). Platelet adenylate cyclase responses in depression. Implications for a receptor defect. *Psychopharmacologia*, 36, 291-300.
- WANG, Y.C., PANDEY, G.N., MENDELS, J. and FRAZER, A. (1974b). Effect of lithium on prostaglandin  $E_1$ -stimulated adenylate cyclase activity of human platelets. *Biochem. Pharmacol.*, 23, 845-855.
- WEIL-MALHERBE, H. and VAN BUREN, J.M. (1969). The excretion of dopamine and dopamine metabolites in Parkinson's disease and the effect of diet thereon. *J. Lab. Clin. Med.*, 74, 305-318.
- WEISSBACH, H. and REDFIELD, B.G. (1960). Factors affecting the uptake of 5-hydroxytryptamine by human platelets in an organic medium. *J. Biol. Chem.*, 235, 3287-3291.

- WERDINIUS, B. (1967). Effect of probenecid on the levels of monoamine metabolites in the rat brain. *Acta Pharmacol. Toxicol.*, 25, 18-23.
- WHITTAM, R. and WHEELER, K.P. (1970). Transport across cell membranes. *Annu. Rev. Physiol.*, 32, 21-60.
- WILK, S., SHOPSIN, B., GERSHON, S. and SUHL, M. (1972). Cerebrospinal fluid levels of MHPG in affective disorders. *Nature*, 235, 440-441.
- WING, J.K., COOPER, J.E. and SARTORIUS, N. (1974). The measurement and classification of psychiatric symptoms. Cambridge University Press, Cambridge.
- WINS, P. and SCHOFFENIELS, E. (1966). Studies on red-cell ghost ATPase systems: properties of a ( $Mg^{2+} + Ca^{2+}$ )-dependent ATPase. *Biochim. Biophys. Acta*, 120, 341-350.
- WIRZ-JUSTICE, A., FEER, H. and RICHTER, R. (1977a). Circannual rhythm in human plasma free and total tryptophan, platelet serotonin, monoamine oxidase activity, and protein. *Chronobiologia*, 4, 165-166.
- WIRZ-JUSTICE, A., LICHTSTEINER, M., FEER, H. (1977b). Diurnal and seasonal variations in human platelet serotonin. *J. Neural Transm.*, 41, 7-15.
- WIRZ-JUSTICE, A. and PÜHRINGER, W. (1978a). Seasonal incidence of an altered diurnal rhythm of platelet serotonin in unipolar depression. *J. Neural Transm.*, 42, 45-53.
- WIRZ-JUSTICE, A. and PÜHRINGER, W. (1978b). Increased platelet serotonin in bipolar depression and hypomania. *J. Neural Transm.*, 42, 55-62.
- WISNIESKY, B.J., PARKES, J.G., HUANG, Y.O. and FOX, F. (1974). Physical and physiological evidence for two phase transitions in cytoplasmic membranes of animal cells. *Proc. Natl. Acad. Sci. USA.*, 71, 4381-4385.
- WOLD, F. (1971). Proteins: systems and methods. In 'Macromolecules: structure and function'. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp 30-50.
- WYNN-WILLIAMS, A.T. (1976). An explanation of apparent sudden change in the activation energy of membrane enzymes. *Biochem. J.*, 157, 279-281.
- YATES, C.M., TODRICK, A. and TAIT, A.C. (1964). Effect of imipramine and some analogues on the uptake of 5-hydroxytryptamine by human blood platelets *in vitro*. *J. Pharm. Pharmacol.*, 16, 460-463.